The Journal of Laboratory and Clinical Medicine

VOL. XVIII ST. LOUIS, MO., OCTOBER, 1932 NO. 1

CLINICAL AND EXPERIMENTAL

SERUM PROTEINS AND GLAUCOMA*

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THE serum proteins play an important rôle in maintaining a fluid equilibrium between the blood and the tissues. Starling[1] showed that the osmotic pressure of the plasma crystalloids had little influence on the fluid distribution as compared with the plasma proteins, because the crystalloids easily pass through the vessel wall with the water. Govaerts[2] confirmed Starling's conclusion and proposed a theory to explain the interchange of fluid between the blood stream and the tissues. He demonstrated that the increase of the hydrostatic pressure or decrease of osmotic pressure of the serum proteins tends to cause transudation and to result in edema. Of the serum proteins, albumin with its smaller molecular weight showed a much greater osmotic pressure per gram than globulin. Therefore a decrease of albumin was more likely to cause edema than a deficiency of globulin.

The osmotic pressure of the blood proteins and crystalloids, and the capillary hydrostatic pressure are probably the chief factors which maintain the normal intraocular pressure. The osmotic pressure of the proteins has perhaps the most constant influence on the intraocular pressure. The hydrostatic pressure of the capillaries has not as yet been measured. There has been a tendency in the past to relate the hypertension in the eye with the lower osmotic pressure of the blood. Elliot[3] reported that Mayard observed 20 cases of glaucoma associated with a wave of epidemic dropsy. Hertel and Citron[4] believed that the total osmotic pressure of the blood of patients with glaucoma was generally less than that of normal persons. An osmotic pressure of over 6000 mm. Hg was found in 16 per cent of the patients with glaucoma, and a pressure lower than 5600 mm. Hg was never observed in cases with normal intraocular tension but

*From the Wilmer Ophthalmological Institute of Johns Hopkins University and Hospital. Received for publication, December 22, 1931.

was present in 45 per cent of glaucomatous cases. Dieter[5] and Serr[6] observed no unusual deviation of the serum osmotic pressure in patients with glaucoma.

Methods.—The patients were allowed to become adjusted to the routine of the hospital. The blood for analysis was always obtained under the same conditions. It was drawn from the antecubital veins without stasis before breakfast after a fast of at least fourteen hours to avoid inspissation of the serum. The blood was collected and serum separated by the means which produced the least change of water content of the serum. The method of Howe as modified by Bruckman[7] was employed for the determination of plasma proteins. The ocular tension was taken by a standard Schiötz tonometer.

Discussion.—The fractionation of the blood serum proteins of glaucomatous patients has not been reported previously. However, others have estimated the total serum proteins by the refractometric method which is known to give higher values than the Kjeldahl method.[8]

TABLE I

SERUM PROTEINS OF GLAUCOMATOUS PATIENTS

NO.	AGE	MEDICAL DIAGNOSIS	TYPE OF GLAUCOMA	TENSION SCHIÖTZ MM. HG RT.	TENSION SCHIÖTZ MM. HG LT.	NPN MG. PER CENT	SERUM PROTEINS		
							TOTAL PROTEIN PER CENT	ALBUMIN PER CENT	GLOBULIN PER CENT
1	57	Normal	O. U. Chronic	26	47	28	6.08	4.26	1.82
2	76	Arteriosclerosis	O. U. Chronic	34	29	34	6.20	4.37	1.83
3	62	Normal	O. U. Chronic	65	32	41	6.20	4.46	1.74
4	57	Ing. hernia	O. U. Chronic	26	25	27	6.24	4.47	1.77
5	58	Arteriosclerosis	O. U. Chronic	29	40	29	6.81	4.35	2.46
6	76	Ing. hernia, arteriosclerosis	O. U. Chronic	50	31	29	7.04	4.25	2.79
7	62	Ing. hernia	O. D. Anophthalmos O. S. Chronic	–	61	33	7.05	4.61	2.44
8	58	Normal	O. U. Chronic	42	45	28	7.07	4.56	2.51
9	69	Hypertension	O. U. Chronic	33	27	31	7.07	4.73	2.34
10	65	Hypertension, arteriosclerosis	O. U. Chronic	29	52	33	7.13	4.22	2.91
11	51	Hypertension	O. U. Chronic	26	28	29	7.14	5.90	1.24
12	64	Hypertension	O. U. Chronic	26	38	31	7.18	4.28	2.90
13	55	Normal	O. U. Chronic	51	32	27	7.27	5.21	2.06
14	82	Arteriosclerosis	O. U. Chronic	74	25	34	7.30	4.73	2.57
15	47	Normal	O. U. Chronic	90	70	24	7.36	4.88	2.48
16	59	Syphilis	O. D. Chronic O. S. Acute	25	51	28	7.36	5.02	2.34
17	69	Hypertension, arteriosclerosis	O. U. Chronic	43	25	29	7.45	4.52	2.93
18	58	Normal	O. U. Chronic	61	29	28	7.61	4.74	2.87
19	63	Hypertension, arteriosclerosis	O. U. Chronic	36	28	22	7.99	5.01	2.98
20	73	Hypertension, arteriosclerosis	O. D. Phthisis-bulbi O. S. Chronic	–	65	35	8.00	5.04	2.96

The value of the serum proteins in glaucomatous patients has a fairly wide range. Repeated determinations the following day showed practically little difference. The total serum proteins varied from (see Table I) 6.08 to 8.00 per

cent, the serum albumin from 4.22 to 5.90 per cent, and the serum globulin from 1.24 to 2.98 per cent. If Bruckman's determinations are accepted as normal values, the serum proteins fall within the normal range. From these data there is no reason to believe that chronic glaucoma is directly associated with the concentration of blood proteins.

Conclusion.—There is no essential quantitative difference between the serum proteins of healthy persons and of patients suffering with an uncomplicated glaucoma.

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THE NONGLUCOSE REDUCING BODIES IN DIABETIC BLOOD*

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THE nonglucose reducing body which makes the reduction with most blood sugar methods too high has already been found constant in health and disease, and specifically in diabetes, by Folin and Svedberg.[1] This paper confirms their report. We estimated the substance in terms of glucose as the nonfermented reduction by the Folin-Wu method after treatment of the whole blood with an equal quantity of yeast suspension for five minutes. This substance, called "glucid X" by Fontès and Thivolle,[2] the "saccharoid" of Benedict,[3] is the reducing substance analysed by Best,[4] in part composed of pentose, which makes the apparent blood sugar by the Folin-Wu method about 20 mg. per cent too high. The amount of true glucose is obtained by subtracting the value for glucid X from the apparent glucose, or the true glucose may be determined directly by the Folin-Wu method on the copper filtrate Somogyi suggests[5] and glucid X estimated as the excess reduction in the tungstate filtrate. As a rule we found the two methods checked closely. It is possible that the copper and tungstate comparison may prove to be the better, since glucid X is sometimes completely fermented in a few minutes (vide infra) and may not infrequently be destroyed in part by five minutes' yeast fermentation.

Our chief interest was in the "Y-reduction" which Pickard, Pierce and associates[6] found constantly as a reduction over that of glucose and glucid X.

*Received for publication, December 4, 1931.

TABLE I

GLUCOSE, GLUCID X AND Y-REDUCTION (GLUTATHION?) IN DIABETES

PATIENT	FW	ION	GLUCOSE	GLUCID X	Y-REDUCTION
1. Re., Dr. Oatman. Sugar free, diet only 3 yr.	156	210	140	16	54
Same, later	132	210	116	16	78
2. Ma., Dr. Potter. No insulin	490	550	484	6	60
3. Br., Dr. Potter	139	206	115	24	67
4. Fa., Dr. Brenner. Diet only. Age 75	178	208	157	21	30
5. Ju. 31, Mercy H.	244	320	221	23	76
6. Be., Dr. Miller	170	234	144	26	64
7. Ho., Dr. Brenner. Blood stood 16 hr. in room	284	290	276	8	6
Same, after 10 U insulin	265	298	253	12	33
8. Bg., Dr. Miller	400	550	385	15	150
4 d. later, 2 hr. after 40 U insulin. In shock. Blood stood room 16 hr.	21	106		8	85
7 d. later, on 20 U daily, blood 2 hr. after insulin	60	128	44	16	68
9 d. later, 40 U daily	122	180	106	16	58
11 d. later, same insulin. Urine, trace sugar	160	185	140	20	25
2 wk. later, same dosage	196	250	186	10	54
9 d. later, same dosage	261	319	225	34	58
3 wk. later. Has gained 15 lbs. Urine trace sugar	135	170			35
9. Dr., Dr. Miller. Diet only	128	206	116	12	78
3 wk. later	120	152	113	7	32
10. Me., Dr. Brenner. Age 60, diet only was 480 glucose	206	198	178	28	0
Later date	280	350			70
11. Fo., Dr. Miller. Diet only, ins. refused	348	390	335	13	42
12. Ha., Dr. Brenner. Diet, no insulin	140	198	127	14	58
14. De., Dr. Kennell. Diet only, 3 yr. sugar free urine	150	250	130	20	100
15. 38-178 Dr. Ball. Insulin 2 hr. previously	304	300	280	20	0
16. 38-181 Dr. Ball. Insulin 3 hr. previously	325	355	310	15	30
Rabbit, 2.5 kg.	127	200	99	28	73
Same, next day, before insulin	105	240	90	15	135
¾ hr. after 15 U insulin	55	145	41	15	90

FW, apparent sugar by Folin-Wu method. Ion, same by Ionesco method. Glucose, true glucose, after subtracting glucid X from FW. All figures mg. per 100 c.c.

Pickard[7] has shown that the Y-reduction is probably due in large part to the glutathione of the cells. We estimated the Y-reduction by subtracting the reduction by the Folin-Wu method (glucose plus glucid X) from the reduction, as glucose, in the same tungstate filtrate, by the Ionesco method.[8] The Y-reduction in normal young people was 40 mg. per cent average;[6] in normal adults it was the same.[7] The average Y-reduction in 26 diabetic blood specimens was 54 mg., varying in a wide range, as was also the case in normal blood specimens. In the diabetic glutathion as an oxygen carrier in the cells might be influenced by the excess glucose in the blood. We found a definite increase in the Y-reduction on the average. Two samples, however, showed no Y-reduction. In case 8 with the highest figure, 150 mg., the Y-reduction became lower with improvement in her condition, and gain in weight; the average of her last 4 examinations is normal.

Since Fontès and Thivolle found no reduction in the tungstate filtrate after fermentation of the blood with yeast, using their reagents, they thought that glucid X was always fermentable completely and immediately, with the true glucose. The loss of sensitivity of their reagents to glucid X after fermentation shows evidence of a change in this substance, preliminary to its destruction. Most writers describe glucid X as the "non-fermentable" non-glucose reduction. Pickard, Pierce and associates found glucid X is occasionally fermented with the true glucose, giving no reduction with the Folin-Wu method; in other bloods it was fermented completely in an hour. We have found that it completely disappears after three days' fermentation (Table II).

TABLE II

FERMENTABILITY OF GLUCID X, "NONFERMENTABLE," NONGLUCOSE REDUCING BODY

PATIENT, TABLE I	TIME OF FERMENTATION. REDUCTION MG. PER CENT AS GLUCOSE						
	5 MIN.	2 HR.	16 HR.	24 HR.	2 DA.	3 DA.	4 DA.
3	24			13			
5	23	16					
8	15		10				
10	28						0
6	26						1
7	12				3		
8	16				3		
St	12			9			
14	20					0	
13	15					0	
11	13				6		

Equal quantities of blood and yeast (Fleischmann) suspension. Room temperature. Ordinary tungstate precipitation, Folin-Wu blood glucose technic.

Besides its fermentability Fontès and Thivolle found glucid X decreased after insulin and in ratio with the glucose. They thought therefore it should continue to be estimated with the blood glucose as a physiologically similar sugar. Table I does not show any relationship of either glucid X or the Y-reduction to the amount of glucose. In 24 blood samples glucid X averaged 16.5 mg. per cent, the normal by the Folin-Wu technic is 20 mg., and Folin and Svedberg found the

same in diabetics. The lowest figure we found for glucid X, 6 mg., is in the blood with the highest glucose. The two bloods in which glucid X was 8 mg. had stood at room temperature for sixteen hours, and we have found that autolysis on standing destroys glucid X in part, as it does the glucose. Glucid X was low in patients who never had insulin.

Since the amount of glucid X does not vary much and in any case is small compared to glucose, there has been little practical result from the confusion in the "normal" figure for blood sugar which has resulted from the introduction of various new methods for glucose, methods which have only approximated the true values. The Folin ferricyanide and the Benedict copper technics give about half the value for glucid X of the Folin-Wu or the Hagedorn-Jensen, perhaps showing only the pentose fraction. We believe that it is desirable to make the estimation of blood glucose exact, now that there is a simple, practical and accurate technic. This technic is the precipitation of the proteins, and of glucid X, by copper, as Somogyi suggests, followed by the Folin-Wu blood sugar method.

1. The substances causing the Y-reduction are increased over the normal in diabetic blood, but vary widely.

2. The "non-fermentable" glucid X is fermented, completely in three days, occasionally it is destroyed with the glucose.

TECHNIC

Copper Precipitation.—One volume blood, lake in 7 volumes water, add 1 volume 7 per cent copper sulphate, shake. Add 1 volume 10 per cent sodium tungstate drop by drop, shaking. Filter.

Ionesco Method.—Put 5 c.c. 1/10 blood filtrate in a 200 by 15 tube, add 2 c.c. N/1 NaOH, 1 c.c. ferricyanide reagent (23 gm. K ferricyanide, 23 gm. KOH, water to 1000), 10 c.c. water. Boiling water-bath twelve minutes, cool, color should be yellow. If colorless repeat using 2.5 c.c. filtrate. Add 5 c.c. 20 per cent Fe free H_2SO_4. Titrate from microburette with N/60 $KMnO_4$ to rose color. Subtract correction of blank. One c.c. N/60 $KMnO_4$ equivalent to 100 mg. glucose per 100 c.c. blood.

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ANGIONEUROTIC EDEMA: ITS RELATION TO BACTERIAL HYPERSENSITIVITY*

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THE suggestion is to be found in most recent discussions of the disease that the peculiar symptom-complex described under the name angioneurotic edema may be included with those phenomena which we crowd under the ever-enlarging canopy of the term allergy. For the most part it is seldom more than a suggestion and definite evidence of a convincing character demonstrating sensitization and the amelioration of symptoms following desensitization is largely lacking. Quincke, whose name has been given to the condition, is responsible for the opinion that the fundamental pathogenic factor is an angioneurosis, whatever that may be. This belief has persisted and would seem to us to have exerted a pernicious influence in inhibiting active inquiry regarding the actual cause. Though the clinical syndrome is a fairly common one and is now generally recognized there has been astonishingly little curiosity concerning its odd manifestations, if we may be guided by the reported studies. Nor can the usual benign character of the disease be accepted as an excuse for this apparent lack of interest because no one can witness the picture of acute suffocation subsequent to edema of the glottis without realizing the frightful potentialities involved whenever the diagnosis of angioneurotic edema is made.

The obvious relationship between the disease and giant urticaria has long pointed in the direction of specific hypersensitivity, and Osler[1] mentions one case in which desensitization with peptone was followed by the relief of symptoms. Mackenzie,[2] in his discussion of this disease, writes as follows: "Recent evidence makes it probable that in some of the nonhereditary cases the condition is due to food allergy." He then drops this phase of the subject without further comment. Wherry,[3] who has been the first to make so many important suggestions, was, we believe, the first to point out the probable causal relationship between bacterial hypersensitivity and angioneurotic edema. Unfortunately, his patient died of an intercurrent infection before he was able to complete the process of desensitization. The evidence he offers, however, is most interesting and it was the stimulation of Wherry's report which directed us to the careful study of a series of cases of angioneurotic edema.

We wish to report in this paper the detailed study of three such cases. These three do not exhaust our series but we prefer to report them in considerable detail and this would be impossible in a larger series. Also the three patients under consideration have all been observed for a period of at least two years *since* desensitization, and we feel that this is the minimal time factor which

*From the Department of Internal Medicine and the Louis Kuhn Diagnostic Laboratory. Received for publication, December 16, 1931.

justifies the drawing of conclusions. All the cases to be reported are of the nonhereditary type and it may be possible that such conclusions as we have reached will obtain only in that form of the disease, though Wherry's case had a decided hereditary background.

This study forms only one small phase of a much more comprehensive inquiry into the field of bacterial hypersensitivity, and it will be necessary to refer very briefly to certain concepts and methods in technic which are more fully elaborated in previous papers.[4, 5, 6, 7] Any one interested in the application of our methods is referred to these reports. We have been especially interested in the possible significance of intradermal tests when employed to indicate specific sensitivity to bacterial antigens. We believe that we have been able to use such tests to great advantage in studying bacterial asthma, arthritis, nonulcerative colitis, and certain skin conditions, and by use of them have demonstrated in such conditions a fundamental bacterial hypersensitivity. Antigens of the bacterial type, selected on the basis of positive skin reactions and given by the "desensitization" method have yielded excellent therapeutic results.

Interested in such problems, it was inevitable that we should include in our studies such a curious disease as angioneurotic edema. We began these studies by attempting to demonstrate the role of food allergy in the etiology of the disease. This was done by the use of intradermal food tests and of "selective restriction" diets. By these means we were able to obtain evidence suggesting food sensitization in two cases and were led to a striking therapeutic result in the management of one of them. In a disappointingly large number, however, we were unable to prove the existence of food allergy, and utilized the methods which we had developed for the study of bacterial hypersensitivity for the further investigation of these refractory cases. It is this second group which we wish to consider in the present paper.

CASE 1.—This patient was a white male, aged thirty-eight, and he gave a history of unusually good health prior to an acute attack of sinusitis which occurred in 1924. We first saw him in October of 1928. He had been having recurring attacks of angioneurotic edema since the spring of 1925. The attacks were rather mild and occurred at two to three-month intervals but they almost invariably involved the face, and the patient was as much distressed by the cosmetic effect as by the actual discomfort. There was also a history of fleeting attacks of "hives" but none of these had lasted longer than from twelve to eighteen hours.

Food tests showed him to be sensitive to cocoa and certain sea foods. These had never constituted an important part of his diet and were eliminated with no apparent therapeutic result. We then directed our attention to the problem of bacterial sensitization. Enteric cultures yielded a number of organisms but he failed to show marked sensitivity to any of them. According to his history the disease had its onset following an acute attack of sinusitis and the x-ray examination of the sinuses suggested a chronic ethmoid infection. There was also a history of postnasal "drip" and headache, both of which post-dated the attack of sinusitis.

Cultures were made from material obtained by suction from the ethmoid region. These cultures yielded a pure growth of hemolytic staphylococcus albus. An antigen prepared from this strain produced a marked local reaction when injected into the skin. Desensitization was carried out beginning with one minim of the vaccine in dilute suspension and slowly increasing the dose by one-half minim intervals to a maximum dose of four minims. Injections were given every second day and were continued for six months. Before the first month had passed he had one mild attack and to date (December, 1931) he has never had

another. This patient responded more readily than any other we have studied and again emphasizes a fact which we have previously pointed out, that the hemolytic staphylococcus is an excellent antigen for use in specific desensitization. There was also marked improvement in the symptoms of chronic sinusitis.

CASE 2.—A white woman of forty-five whose history of urticaria and angioneurotic edema was of six years' duration. The disease most frequently exhibited itself as a giant urticaria but upon frequent occasions there was swelling of the ankles and feet and occasionally of the lips. As there was no financial handicap to the search for relief this patient had been studied in several clinics of distinction, both in the east and in the middle-west. Dietary restrictions had been attempted but had given no permanent relief and "food tests" had been carried out on several occasions but had failed to give information of value in treatment. The attacks were, in general, cyclic with excessive fatigue, nervousness, and excitement acting as precipitating factors. Following complete rest and a change of environment there had been one distinct intermission of months during which she was practically symptom-free. This was followed by a reexacerbation of symptoms more exaggerated than ever and when we first interviewed the patient she was suffering with almost daily attacks of urticaria and frequent seizures of the true angioneurotic type.

In our study of the problem we could make no case for the probability of a food sensitization. We reviewed the previous careful studies and these as well as our own investigations failed to convince us that we were dealing with food allergy. The possibility of a bacterial sensitization of the enteric type was suggested by both the history and the physical examinations. The history was that so often given by patients with an "irritable colon," a condition which we have previously discussed in its relationship to bacterial hypersensitivity.[4, 5] Upon examination the distal colon was distinctly spastic and tender, the proximal colon definitely distended. Hence, we directed our attention to the study of the intestinal flora.

Stool cultures yielded the following organisms: (1) B. coli, strongly hemolytic; (2) B. coli, nonhemolytic; (3) B. mucosus capsulatus; (4) streptococcus of indifferent cultural reactions; and (5) Staphylococcus albus, nonhemolytic. The predominating organism in the fecal flora, however, was the hemolytic colon bacillus and the lysis shown when this organism was grown upon blood agar plates was remarkable. Intradermal tests were run using each of the five strains as antigens. The hemolytic B. coli gave rise to a marked positive reaction with erythema and edema at the site of injection. There were very moderate reactions to the nonhemolytic colon bacillus and to B. mucosus capsulatus while there was no reaction to the other strains. We selected strain 1 as that most distinctly indicated by the skin reactions and further tested the causal relationship of this organism by giving a two minim intradermal dose of an autolysed filtrate. This proved to be a most interesting and enlightening experiment, for within two minutes of the injection there was marked local edema and within five minutes the patient complained of numbness and tingling of the upper lip and, under our eyes, developed a typical angioneurotic swelling of the upper lip and right cheek. This was controlled with adrenalin. Here we were able to produce not only local swelling by the intracutaneous injection of an autolysed antigen, but what is most convincing, a focal reaction as well.

The treatment consisted in desensitization by using heat killed suspension of the hemolytic B. coli as the antigen. A dilute saline suspension was employed and the treatment started with the intracutaneous injection of one minim. This dose was continued every second day until eight injections had been given and was then increased to one and one-half minims. From this point the dosage was slowly advanced by one-half minim intervals and each interval was maintained for from four to six injections. Five minims was the maximum dose and when reached, was continued. The vaccine was given over a period of three and one-half months and then discontinued. During the first two weeks of the treatment there were two mild attacks, then freedom for five weeks, the longest period without an attack in months. There was then a rather sharp attack with swelling of the face which followed an attempt to advance the dose of vaccine too rapidly. This was readjusted and the process of desensitization continued as outlined. From this time on the symptoms diminished in severity very rapidly. There were scattered mild attacks

of urticaria but no major swelling and after a few months these stopped entirely. After four months of treatment the skin sensitivity had practically disappeared. A year later the patient was retested with the antigen and found to be again moderately sensitive, so she was given two minims of vaccine every second day for two months. During this interval of secondary treatment there were no skin symptoms and when tested for sensitivity nine months later the reaction was negligible. She has now been free of urticaria and angioneurotic edema for more than two years.

CASE 3.—This patient was a white woman of forty who presented the usual symptoms of swelling of the face, hands, and feet which came on rapidly, lasted about twenty-four hours and then, as suddenly, disappeared. On several occasions she had experienced severe gastroenteric crises which were associated with the attacks. Previous to the actual swelling there would often be premonitory symptoms consisting in a feeling of distinct warmth and itching in the particular area where the swelling was to appear. It is interesting to note that this patient gave a history of asthmatic attacks which followed influenza in 1918 and which, apparently, were self-limited since they ceased within two years and have not recurred.

The initial attack of angioneurotic edema occurred early in 1925 and came on after a prolonged exposure to water and cold. We first saw the patient in December of 1927 so the condition had been established for almost three years. At first the attacks had been infrequent but after the first year the interval of remission had shortened rapidly and two or three attacks each month had been the story for the last twelve months before we studied her case.

The general physical examination was essentially negative with the exception of a distinctly spastic distal colon and she gave a history of chronic constipation of years' duration. When studied for sensitivity to food proteins she was found to react to egg albumen, milk, fish, and nuts. Elimination of these foods from her diet did not in any way influence the course of her disease.

From her stool cultures we recovered a Staphylococcus aureus and Streptococcus hemolyticus, B. cloacus, and B. coli communior. When skin-tested to these various strains in pure culture, she was markedly sensitive to the B. coli communior. Similarly tested to a nonautogenous strain of the same organism with which we happened to be working at the time, she gave a mild reaction in no way comparable with that caused by the autogenous strain. Treatment with the "active strain" was started in January of 1928. The first injection was given employing one minim of a fairly heavy suspension and was immediately followed by an acute attack of angioneurotic edema. This necessitated a dilution of 1:5 and she tolerated a one minim dose of this suspension without untoward results. We were able to continue with this dose, giving it every second day, for three weeks. Increased dosage, at this point, again brought on fresh manifestations and during an interval allowed to pass while the vaccine was being further diluted, the patient had a spontaneous attack associated with acute edema of the glottis which necessitated hospitalization and the closest observation. We mention this fact because certain writers have been inclined to consider the nonhereditary form of the disease as benign and to associate edema of the glottis with the hereditary type alone. Such a point of view is contrary to fact, and we would emphasize the statement that edema of the glottis may occur in either type.

Desensitization was resumed using very minute doses of a quite dilute suspension and after several weeks we were able to increase the dose cautiously without reactions. The process was a tedious one but gradually the attacks diminished in severity and intensity. In June, 1928, a fresh specimen of stool yielded the same organism and in addition a strain of B. mucosus capsulatus to which the patient was also quite sensitive. This was added to the vaccine and the desensitization resumed, the maximum dose employed being four minims. She continued to have mild attacks through the early fall of 1928 but was so encouraged by the distinct change in the character of the seizures that she cooperated willingly in the tedious treatment. Her skin sensitivity was at this time markedly decreased and through the following winter she was almost free from symptoms. Treatment was continued until August of 1929 and then she was discharged and told to return in

the event of a recurrence of her former trouble. She was last interviewed in November, 1931, two and one-half years since her last mild attack and there have been no recurrences.

DISCUSSION

We have presented, in considerable detail, a study of three cases of angioneurotic edema in which we were unable to demonstrate the causal relationship of food sensitization. We were able, in each case, to show what we have come to regard as definite evidence of bacterial hypersensitivity and by an adequate course of desensitization in which selected antigens were used, we have kept each of these patients symptom-free for more than two years. The first case presented a rather simple problem and the course of desensitization was not an arduous one. Cases 2 and 3, on the other hand, illustrate very well the difficulties encountered when one attempts to desensitize susceptible patients with bacterial antigens. At the same time they demonstrate the value of careful, persistent effort in this direction. We have previously called attention to the great value of vaccine therapy when conducted in this manner and wish again to emphasize the fact that desensitization of an already sensitized individual is one problem and the immunization of a noninfected individual is distinctly another. We are certain that, had vaccine been given to these individuals in massive doses they would not have improved, rather the converse would have resulted. We have seen the exaggeration of symptoms follow the use of large doses of vaccine not only in angioneurotic edema but also in asthma, nonulcerative colitis and a certain type of joint effusion. We are confident that the failure to take this factor into account is responsible for many of the failures in vaccine therapy. The other great source of disappointment is the failure to select "specific" autogenous antigens to which the patient is sensitive.

CONCLUSION

We believe that we have demonstrated the importance of bacterial hypersensitivity as an etiologic factor in angioneurotic edema.

Adequate desensitization is associated with the disappearance of symptoms.

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THE ALLEGED CLINICAL INTESTINAL ACTIONS OF APOCODEINE*

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IT HAS been reported that apocodeine is an efficient and valuable clinical cathartic, which can act orally and hypodermically. The claims for the cathartic action appear to be founded on certain experimental results which seemed to show that the action of apocodeine is mediated through depression or paralysis of the sympathetic ganglia, thus leaving the intestine to parasympathetic nerve control. If true, the advantages of such a drug would be obvious in conditions accompanied by over-stimulation of the sympathetic nervous system, as in the so-called sympathicotonia and related nervous disorders, in postoperative paralytic ileus, in the paresis of peritonitis, etc.

Perhaps the last, and also most convincing, clinical report is that of W. C. Alvarez,[1] who reported that the oral administration of apocodeine in doses of 1/5 to 1/10 gr. (4.4 to 6.5 mg.) orally caused one to three normal evacuations of the bowels daily; generally, the drug was given together with atropine. Some of Alvarez's patients continued the use of apocodeine for as long as three years: there was neither habituation, nor need to increase the dosage. Several years previously, Guinard,[2] Combemale[3] and Toy[4] all reported favorably upon the cathartic action of apocodeine. The two former reporters used the drug hypodermically; the latter employed it orally as a sedative for psychotic patients, and observed the cathartic action only incidentally.

The only other clinical use claimed for apocodeine is as an expectorant, reported by Murrell[5] in 1871, and by Fröhner[6] in 1893. This could be the result of a nauseant action, not infrequently caused by the apocodeine, which chemically resembles apomorphine. Our own experiences with apocodeine agree with the claims of Legg,[7] who, in 1870, found no effect on man using the drug in doses ranging from 1 to 3 gr. (65 to 195 mg.).

Concurrently with our clinical trials, Dr. E. W. Schwartze[8] reinvestigated the important pharmacologic actions of apocodeine. Schwartze was unable to confirm the claim that apocodeine acts as a paralyzant for sympathetic ganglia of the gastrointestinal and circulatory systems. Excised intestinal musculature was depressed by the drug, and the cathartic action in intact animals was found to be "variable and indefinite." He failed to observe any erection of the hairs, or redness and edema of the face, although the doses of apocodeine employed were sufficient to cause considerable circulatory depression. A certain, though fleeting, increase in intestinal peristalsis accompanied the circulatory depression, but only at the expense of such depression, and the intestinal action could be recapitulated with a circulatory depression of mechanical origin. Emesis and defecation occurred in dogs in about the same way as after morphine; the seat of these actions was presumably central, and not ganglionic or in the intestine.

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Received for publication, December 23, 1931.

Schwartze could demonstrate a paralytic, nicotine-like action of the apocodeine on the ganglia of the cardiac vagi, but this is irrelevant to a consideration of the intestinal and cathartic actions.

Methods.—Apocodeine hydrochloride was used by us in a total of 22 patients. Three of these patients suffered from postoperative ileus. The remaining 19 patients had apparently normal gastrointestinal tracts; four had entered the hospital for cystoscopy and pyelograms; one came in for dilatation of the ureters; one had pyelitis, and the remaining 16 were normal obstetric patients one week postpartum. All the patients were confined in bed at the time of our investigation. The apocodeine was used in aqueous solution given intramuscularly in the gluteal region.

The doses of apocodeine in our patients varied from 12 to 97 mg., and the same three products of the drug used by Dr. Schwartze in his pharmacologic investigation were used by us. Two of these samples of apocodeine (Merck) were dark green in color, amorphous and presumably impure. One other sample was specially prepared for our use by Merck and Company; it was white, amorphous and presumably purer than the green products.

Control readings of blood pressure, pulse and respiration were taken at fifteen-minute intervals during one hour before the administration of the drug, and during three hours afterwards. The patients were closely observed for changes in diameter of the pupils, for presence of colic, nausea and defecation, for relief of pain where pain was present, and for subjective symptoms. The data obtained were tabulated and summarized, but the table is omitted as the net result for our purpose was practically negative; an abstract of the essential results is here presented.

General Effects.—In two (9 per cent) patients (Vr and Rs) slight constriction of the pupils occurred. Colic of slight and transient character occurred in one (4.5 per cent) patient (Hh). Nausea occurred in 6 patients (Hh, Wd, Li, Rs, Ly and Kg) or 27.2 per cent, and of this number 4, or about 66 per cent (Li, Rs, Ly and Kg), vomited. Defecation did not occur in any of the patients. In three patients (Cr, Ht and Rs), a slight narcotic action with relief of pain was observed. In two patients (Ge and Rs), who had severe pain following ureteral catheterization, the apocodeine failed to give any relief after three hours, and morphine sulphate had to be used.

All three samples of apocodeine caused more or less local irritation after intramuscular injection. The irritation after the dark green apocodeine was comparatively negligible, but the white, and supposedly purer, product gave such severe local reactions that its use was discontinued. The injection areas of the three patients treated with white apocodeine threatened to break down and form abscesses and all three patients experienced systemic reactions, including fever up to 38° C.

Circulatory Effects.—In no patient did the apocodeine produce any notable change in blood pressure, pulse rate, or respiration. The circulatory depression, commonly observed in laboratory animals, did not occur, probably because the dosage of the drug was not sufficient, and it was not used intravenously as was the case in animals. The widest variation in blood pressure from the mean was 5 per cent, and that in the pulse, 7 per cent.

Failure of Apocodeine in Postoperative Ileus.—The action of apocodeine was closely followed in three patients with paralytic ileus. Patient Wo developed ileus following a hysterectomy and appendectomy; forty-five minutes after the injection of 16 mg. apocodeine, the patient complained of nausea, but did not vomit. Five hours after the injection there had been no colic, and no passage of flatus or feces. At this time, 40 c.c. of 20 per cent sodium chloride solution were given intravenously and soon afterward the patient passed flatus, and peristaltic sounds were audible through the abdominal wall. Patient Ly, who suffered a partial ileus as the result of a pyelitis, received two doses of apocodeine (11 mg. each), given four hours apart, and a third dose of 16 mg. eight hours after the second dose. The patient was nauseated and and vomited after the second dose. There was no improvement in the ileus until thirty-four hours after the last dose of apocodeine, that is, long after its use had been abandoned, and other measures adopted. Patient Kg developed ileus following the operative relief of an intestinal obstruction. She was given two doses of apocodeine (each 11 mg.) within six hours. There was considerable nausea and the patient vomited twice, but there was no colic, or passage of flatus or feces. The ileus was overcome forty-eight hours later by vigorous treatment of the patient with hypertonic sodium chloride solution intravenously and pituitary extract hypodermically.

Comment.—In our hands three specimens of apocodeine did not exert any demonstrable clinical effects on the intestine, as indicated by passage of flatus and feces in 22 patients. There were no cathartic actions, no increase in peristaltic sounds and no colic, or other evidences that peristalsis was increased. The nausea and vomiting following the larger doses appeared to be of central origin, as in animals. In none of the patients were there any signs or symptoms which could be attributed to depression or paralysis of the sympathetic nervous system. Occasionally, the drug seemed to exert a mild hypnotic effect. Since the drug was administered systemically (intramuscularly) in all patients, the essentially negative results as to catharsis and related or accompanying effects could not be attributed to lack of absorption, as might be the case with oral administration. In our patients, the actions of the drug were not assisted or complicated by other drugs.

CONCLUSIONS

1. Apocodeine administered intramuscularly in doses varying from 12 to 97 mg. to 19 normal patients, and to 3 patients with postoperative ileus, had no demonstrable effects on the motor functions of the intestine: increase in peristalsis, as indicated by passage of stools, flatus, and increase in peristaltic sounds, was not demonstrated, and there was no cathartic action. There were indications of nausea and emesis in 27 per cent of the patients; occasionally also of narcosis. These essentially negative results on the alimentary tract under clinical conditions agree with recent negative pharmacologic results in animals.

2. Severe local reactions occurred after intramuscular injection of two apocodeine preparations, a green product and a specially prepared white product, thus indicating the irritant properties of the alkaloid, which according to other evidences, appears to be ill-defined chemically.

3. According to the pharmacologic and clinical results obtained, the clinical therapeutic use of apocodeine, as available at present, at least, appears unwarranted.

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DETOXIFICATION WITH SPECIAL REFERENCE TO SODIUM RICINOLEATE. I.*

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THE detoxification of bacterial toxins and other toxic material is a property which has been attributed by various authors to a great variety of compounds. Among these, sodium ricinoleate is at present being used clinically for a variety of purposes. The present work has been undertaken to study the relationship between chemical structure and detoxifying power, the first step of which has been to investigate the comparative detoxifying activities of sodium ricinoleate and the other products found in a crude castor oil soap. Comparatively little work has been done upon the detoxifying power of pure soaps, so that no quantitative relationships have been established. In most cases, the soap has been prepared from an oil and is referred to merely as a soap of that oil. Even where reference is made to purified soaps, the purity is questionable. In the case of sodium ricinoleate, a study of the various methods of purification which have been published prior to the method described by the writer, raises doubt as to whether or not a truly pure sodium ricinoleate has ever before been prepared.

Davison[3] has studied the detoxifying action of many soaps and reports that their activity decreases in the following order:

1. Castor Oil Soap
2. Coconut Oil Soap
3. Linseed Oil Soap
4. Cottonseed Oil Soap
5. Palm Oil Soap
6. Olive Oil Soap
7. Lard Soap
8. Peanut Oil Soap
9. Corn Oil Soap
10. Sesame Oil Soap
11. Maize Oil Soap
12. Apricot Oil Soap

*From the Research Laboratories of the Wm. S. Merrell Company, Cincinnati, Ohio.

The length of this list, every member of which Davison found to have some detoxifying power, makes necessary the conclusion that detoxification is a more or less general property of soaps, even though the actual detoxifying powers vary greatly.

Nélis[20, 21] believes that sodium oleate behaves toward diphtheria toxin like a catalyst in the presence of which the toxin is definitely destroyed. Raubitschek and Russ[24] found sodium oleate to have an antitoxic effect on neurotoxins, and reported that this action is inhibited by serum, gelatin, and albumoses. Renaud[26] detoxified cobra venom by mixing it with solutions of sodium oleate, sodium palmitate or medicinal soap, the antigenic properties remaining undestroyed. Sédallian and Velluz[30] also noted the rather general occurrence of detoxifying ability among soaps and reported that the presence in the soap molecule of ethylene linkages and of an alcoholic hydroxyl group accentuated this property. Renaud[25] also called attention to the greater detoxifying power of sodium ricinoleate as compared to other soaps. Velluz[31] found that strychnine combined with various soaps to give atoxic compounds, and that metal salts such as mercuric chloride were similarly rendered innocuous by soaps.[32] Vincent[33, 34, 35, 36] reported on the detoxifying powers of many soaps.

This paper does not attempt to give a critical analysis of the material on detoxification by soaps, since there is very general agreement that the property of rendering bacterial toxins atoxic is widespread, and good agreement also that sodium ricinoleate is the most active of the list in this respect.

The only claims against this generality of action were advanced by Larson[16] who states that soaps of oleic, stearic and palmitic acids have no detoxifying action whatever on bacterial toxins and that the presence of these soaps deprives sodium ricinoleate of its detoxifying power.

Sodium ricinoleate is prepared in crude form by the saponification of castor oil. A study of the preparation of a purified product must be based upon a knowledge of the composition of castor oil, since such a knowledge alone gives the necessary information as to the impurities which must be removed. A recent paper by Eibner and Münzing[4] assigns the following percentage composition to medicinal castor oil:

Ricinoleic acid	80
Oleic acid	9
Linolic acid	3
Dihydroxystearic and stearic acids	3
Glycerol and unsaponifiable matter	5

Little agreement is found in other estimates of composition. Krafft[14] discovered that when castor oil was chilled, 3 to 4 per cent of solid glycerides were deposited, in which he found stearic acid. Chonowsky[2] obtained similar results upon chilling an alcoholic solution of castor oil. Juillard[12] discovered about 1 per cent of dihydroxystearic acid. Hazura and Grüssner[10] found that the oxidation of the liquid fatty acids gave two isomeric trihydroxystearic acids, and for this reason they believed that castor oil contained two isomeric ricinoleic acids. They found no oleic acid. Mangold[18] later showed that one unsaturated fatty acid may yield two isomeric hydroxy acids. Haller[8] found no isoricinoleic acid and no oleic acid. Fahrion[5] found 2 to 3 per cent of linolic acid and 3 per cent of oleic acid. Lifschütz[17] has described a spectroscopic

method for the recognition of oleic acid in the presence of other fatty acids, and by application of this method to a study of the minimal concentrations of oleic acid and of castor oil which gave the characteristic spectrum, has estimated that the oleic acid content of castor oil is 0.6 to 0.8 per cent. In summary the composition as given by Eibner and Münzing is probably qualitatively correct, although there is reasonable doubt as to the quantitative accuracy.

Records of the preparation of castor oil soap go back to Rimmington[28] who in 1871 published a description of the saponification of castor oil. Sayre[29] saponified castor oil with sodium hydroxide and salted out the resulting soap with brine. Neison,[19] Heylmann,[11] Brown,[1] and Gordon[6] followed essentially the same procedure. Larson, Cantwell and Hartzell[15] saponified castor oil with an excess of alcoholic sodium hydroxide, neutralized the excess alkali with hydrochloric acid, filtered and threw the alcoholic soap solution into brine to remove the glycerol. The precipitated soap was washed with brine and dried.

The earliest attempt to prepare a purified sodium ricinoleate was that of Krafft,[14] who in 1888, cooled the free fatty acids to 0° C. and pressed the solid fatty acids at gradually rising temperatures, obtaining a solid acid melting near room temperature. Juillard[12] later showed that this method gave a product containing stearic acid and dihydroxystearic acids. Juillard attempted the purification of ricinoleic acid by filtering the crude acids to remove the insoluble solid fatty acids, then prepared the barium soaps of the liquid fatty acids and recrystallized them from alcohol. Decomposition of the barium soaps with hydrochloric acid gave a fatty acid product melting at 4-5° C. Haller[8] alcoholized castor oil with methyl alcohol and fractionated the resulting esters before resaponifying. Netter, André, Césari and Cotoni[22] followed Haller's method and in addition purified the resulting fatty acids by the recrystallization of their barium salts from alcohol.

Kozlowski[13] and Halvorson[9] purified the barium soaps by repeated recrystallization from alcohol. Each fraction treated separately, a fraction of sodium soaps was obtained which had the theoretical iodine number for ricinoleic acid. This fact alone is not a complete criterion of purity, however, since a combined contamination with saturated fatty acids and the more unsaturated linolic acid might also give a product with the theoretical iodine number.

Fahrion[5] extracted castor oil and various ricinoleic acid derivatives with petroleum ether, and at a later date Panjutin and Rapoport[23] prepared a partially purified castor oil by extraction with benzine.

In checking the various methods outlined above for the preparation of a pure sodium ricinoleate, the writer found none of them to be satisfactory. The first problem was to remove the saturated stearic and dihydroxystearic acids. The chilling of an alcoholic solution of castor oil and the extraction of castor oil with petroleum ether produced some purification, but were not investigated in great detail, since it is impossible to determine, a priori, whether or not there exist in castor oil mixed glycerides which would retain a portion of the contaminating acids in the purified fraction. Chilling of the free fatty acids to a temperature which would precipitate all of the solid acids possible gave such a viscous liquid that it was impossible to filter it. The classical method for separating saturated and unsaturated fatty acids was then tried, namely,

the ether extraction of the lead salts. When the ether soluble lead soaps were decomposed by hydrochloric acid, the free fatty acids obtained had a high iodine number, and it was believed that all of the saturated acids had been removed. When the sodium soap was prepared, however, its aqueous solutions were found to cloud slightly on chilling. This cloud formation on chilling of a dilute soap solution was found by the writer to be a much more accurate test for the presence of saturated acids than a determination of the iodine number, since the latter cannot be corrected for the unknown amount of linolic acid present.

The purification effected by recrystallization of the barium soaps from alcohol was studied next. A hot alcoholic solution of the mixed barium soaps from the fatty acids of castor oil was cooled gradually to room temperature when more than half of the barium salts had precipitated. The solution was then filtered and further chilled. The last fraction of barium soaps to precipitate was decomposed with acid and it was found that the resulting fatty acids not only had a lower iodine number than those obtained from the ether-extracted lead soaps, but that an aqueous solution of the corresponding sodium soaps clouded badly.

While it is not impossible that these methods of purification if frequently repeated might eventually succeed in removing all of the saturated fatty acids, they are obviously not very satisfactory. While the pure lead soaps of a saturated acid may be insoluble in ether and the corresponding pure barium soap may be insoluble in cool alcohol, both products are soluble to an appreciable extent in the respective solvents containing large amounts of the corresponding salts of ricinoleic acid.

The methyl esters of the combined fatty acids from castor oil were prepared and fractionation attempted. Little success was had, however, since the distillation had to be carried out in vacuo and the boiling points of all of the pure methyl esters are within a few degrees of one another. The combined methyl esters distilled together over a very small temperature range, so that fractionation was practically impossible.

It was finally found that by dissolving the fatty acids in an equal volume of 95 per cent alcohol and chilling, the solid acids precipitated almost quantitatively, and left a supernatant solution which could be easily filtered. A soap prepared from the fatty acids purified as described above gave a solution which remained clear even when frozen.

The solid fatty acids thus separated amounted to 2.6 per cent of the total. After various attempts it was found that the stearic acid could be separated from the dihydroxystearic acid by ether extraction. The ether soluble acids thus separated melted at 65-68° C., and thus were comparatively pure stearic acid (M. P. 69.3°). The ether insoluble material melted at 190-210°, and contained few free acid groups. It is probable that this compound is a lactone of dihydroxystearic acid, since saponification with alcoholic potassium hydroxide and precipitation of the free acid with a mineral acid gave a compound melting at 141°, the correct melting point for dihydroxystearic acid. By separation of aliquots of the two acids, it was found that dihydroxystearic was present to the amount of 1.68 per cent and stearic acid 0.7 per cent of the original castor oil used.

An analysis of the liquid fatty acids after complete removal of the solid fatty acids should yield information as to the relative amounts of ricinoleic, oleic, and linolic acids present. A determination of the acetyl number would give direct evidence as to the amount of ricinoleic acid, since no other hydroxylated acid would be present. The iodine number would give an estimate of the total unsaturation. The unsaturation attributable to ricinoleic acid could be calculated, since it would be in a definite ratio to the acetyl number. The relative amounts of linolic acid and oleic acid present could be then calculated from a knowledge of the unsaturation in the sample attributable to them and their respective unsaturations.

Grün[7] found that when ricinoleic acid was boiled with acetic anhydride, instead of the expected acetylated ricinoleic acid, a compound was obtained, in part, in which two molecules of ricinoleic acid and one of acetic were combined. For this reason, instead of carrying out the suggested analysis on the free fatty acids, the methyl esters were prepared, and an analysis of the same type was performed. The completion of this study as outlined, together with the completion of the experimental knowledge of the amounts of saturated acids and an estimation of the amounts of glycerol and unsaponifiable matter leads to the following percentage composition for castor oil.

Glycerol and unsaponifiable matter	5
Dihydroxystearic acid	2
Stearic acid	1
Ricinoleic acid	85
Linolic acid	6
Oleic acid	1

This cannot be considered an exact analysis, but the writer believes that it is as accurate as any other analysis available.

From this it would appear that the soap prepared from the fatty acids purified by removal of the saturated acids would be approximately 94 per cent pure sodium ricinoleate, together with 5 per cent sodium linoleate and 1 per cent sodium oleate. Linolic acid oxidizes in the air, and due to this the product which has been manipulated acquires a red-brown color. This color can only be removed by a complete removal of the linolic acid.

A complete purification of ricinoleic acid is possible only by a method capable of removing linolic and oleic acids in addition to the saturated acids. As mentioned above, among the unsaturated fatty acids ricinoleic is the only one which contains a hydroxyl group. While the various methyl esters distill together without separation, it is possible to materially raise the boiling point of methyl ricinoleate by acetylation. Since the contaminating acids will be unaffected, the methyl ricinoleate may be separated by fractional distillation in vacuo.

Such a preparation was carried out, and the resulting methyl acetyl ricinoleate was proved to be absolutely pure, since *both* iodine and acetyl numbers were identical with those calculated for the pure product.

From this material a chemically pure sodium ricinoleate was prepared, probably, for the first time. The chemical details of this purification have been published by the writer.[27]

THE COMPARATIVE DETOXIFYING ACTIVITIES OF SODIUM RICINOLEATE OF VARYING DEGREES OF PURITY

This part of the work represents an attempt to determine the effect of concomitantly occurring impurities upon the detoxifying activity of sodium ricinoleate of various degrees of purity. The first sample considered was a sample of crude castor oil soap, which has undergone no purification. This soap has approximately the following percentage composition, based on dry weight.

Sodium ricinoleate	90
Sodium oleate	1
Sodium linoleate	6
Sodium stearate	1
Sodium dihydroxystearate	2

Larson[16] claimed that sodium oleate and sodium stearate have no detoxifying powers whatever, and that they detract from the detoxifying powers of sodium ricinoleate. Many other authors attribute detoxifying power to sodium oleate. The effects of sodium linoleate and sodium dihydroxystearate have not been reported.

A quantitative comparison of the detoxifying activity of the crude soap of the above composition with that of chemically pure sodium ricinoleate will tell the extent to which the contaminating acids effect detoxification. Examination of the detoxifying power of a partially purified soap will give further information on this subject. The soap prepared by removal of the solid fatty acids will have approximately the following percentage composition:

Sodium ricinoleate	93
Sodium oleate	1
Sodium linoleate	6

Thus a comparison of this partially purified soap with the crude soap will show the effect of the saturated stearic and dihydroxystearic acids, while its comparison with the chemically pure soap will show the effect of the unsaturated contaminating acids, oleic and linolic.

A study of the detoxifying powers of each of the contaminating acids themselves in a pure form will also add to knowledge of their effects. Chemically pure samples of the sodium salts of stearic, dihydroxystearic, oleic and linolic acids were prepared. Sodium stearate is almost totally insoluble in water so that there was not the opportunity of studying the detoxifying power of this pure soap in a manner comparable to the study on sodium ricinoleate.

Sodium dihydroxystearate is only slightly soluble in water at room temperature, but a supersaturated solution was prepared with a concentration of 0.15 per cent. This solution has no detoxifying power, at least of the order exhibited by sodium ricinoleate, since 50 M. L. D. of tetanus toxin dissolved in one cubic centimeter of such a solution killed guinea pigs as rapidly as the untreated toxin.

Pure sodium oleate and pure sodium linoleate solutions in concentrations of one per cent and one-half per cent did show a definite detoxifying activity.

Before discussing the quantitative results it is necessary to discuss the method of testing. The minimum lethal dose of the writer's supply of tetanus toxin was accepted as approximately one-tenth of a milligram for a guinea pig of 250 grams. One hundred times this amount was used for each detoxification

test, dissolved in each case in one cubic centimeter of the fluid to be injected, each solution containing a definite concentration of the soap to be tested. This large dose of toxin was used in order to avoid errors due to the weighing of small amounts, and also to avoid so far as possible errors due to the varying activity of various portions of the dried toxin, which in all probability was not entirely homogeneous.

It is possible that the concentration of the soap is the determining factor in detoxification, not the absolute amount present. For this reason, the concentration of the toxin was kept constant in every case, and the concentration of the soap was altered in the various solutions prepared.

The results, in so far as the guinea pig is concerned may be of one of three types: (1) no protection, i. e., death will occur as rapidly as if no soap were present, (2) complete protection, i. e., the pig will live until death from some other cause intervenes, and (3) partial protection, i. e., the pig will not die as rapidly as if no soap had been mixed with the toxin, but will die from tetanus after a somewhat longer interval.

Due to the unknown length of time which a pig would have to be watched to report that the dose had proven absolutely atoxic, the simplest comparative tests are obtained by determining the prolongation of life, compared to a control animal which received straight toxin, of an animal receiving the toxin with a definite concentration of soap. The results found in this quantitative study of the detoxifying powers of the various soaps are summarized in the following tables. The length of the line of crosses in each case represents the average length of life of guinea pigs which had been injected with 100 M. L. D. of tetanus toxin mixed with the designated concentration of the soaps. The final cross signifies the time of death. Each case shown represents the average results of experiments on at least three guinea pigs, since in no animal work can the results be considered typical unless they are consistent in the cases of at least three different individuals.

A series in which the soap concentrations were 0.1 per cent gave partial protection to a very slight degree. In this series there is a slight indication that the C. P. sodium ricinoleate is a more powerful detoxifying agent than the less pure samples, but the differences are very slight. The results are represented graphically as described, each cross representing a period of two hours:

Sodium Ricinoleate 0.1%

Soap Used	Period of Survival
None—control	XXXXXXX
Crude	XXXXXXXX
Purified	XXXXXXXXX
C. P.	XXXXXXXXXX

The same general results are found in the series of pigs injected with the toxin modified by solution in 0.2 per cent soap solutions. This group may be represented graphically as follows, omitting the first part of each line.

Sodium Ricinoleate 0.2%

Soap Used	Period of Survival
None—control	X
Crude	XXXXXXXXXXXXXXXXXXXX
Purified	XXXXXXXXXXXXXXXXXXXXXX
C. P.	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

Here the differences in the results with the soaps of the three degrees of purity are more marked. It must be remembered, also that the crude sample only contains nine-tenths of the sodium ricinoleate in the corresponding C. P. sample, and that if the soaps composing the other tenth were totally inactive, we should expect only nine-tenths of the activity of the pure soap to be apparent in the crude soap. Larson's claims that such soaps as sodium oleate and sodium stearate materially detract from the detoxifying powers of sodium ricinoleate cannot be valid for the concentration in which they are found in the crude castor oil soap, since there is no marked increase in activity when they are removed.

In experiments with the soaps present in higher concentration, the same results are borne out. Pigs receiving 100 M. L. D. tetanus toxin mixed with 0.25 per cent of the C. P. soap survived on the average for 147 hours, while those with the same concentration of crude soap died in 102 hours.

Where 0.3 per cent soap was used, the pigs in the series where the crude soap was used all showed signs of tetanus and two-thirds of them died between the seventh and eighth day. In the corresponding experiments where the C. P. soap was used the animals showed no signs of tetanus and all were alive after eight days. At the end of this time several animals protected by still larger concentrations of soap had died without tetanic symptoms, so that the results from here on cannot be considered accurate.

It has already been stated that sodium dihydroxystearate and sodium stearate do not compare with sodium ricinoleate as detoxifying agents. Sodium oleate and sodium linoleate in 1 per cent solution both give perfect protection to pigs. The following table will give an idea of the comparative quantitative detoxifying powers.

SOAP	CONCENTRATION	DEATH AFTER
Controls		16 hr.
Sodium	1.0%	..
Oleate	0.5%	35 hr.
Sodium	1.0%	..
Linoleate	0.5%	80 hr.
	0.3%	41 hr.
	0.2%	24 hr.
Crude	0.5%	..
Sodium	0.3%	7 days
Ricinoleate	0.2%	54 hr.

SUMMARY

Crude castor oil soap consists of sodium ricinoleate contaminated with sodium oleate, sodium stearate, sodium dihydroxystearate and sodium linoleate. The two saturated compounds, i. e., the stearate and dihydroxystearate do not have detoxifying powers, at least not of the degree found in sodium ricinoleate. They are present in only small quantities, and their removal gives a product of definitely but slightly increased detoxifying powers. Sodium oleate and sodium linoleate both have definite detoxifying powers, which are, however, considerably less than those of sodium ricinoleate. Additional purification by the removal of these soaps again gives a definite but slight increase in activity. There are however, manifold other advantages to purification which need not be discussed at this time.

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BLOOD IODINE STUDIES

I. The Quantitative Determination of the Iodine Content of Blood*

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THAT there exists an interrelationship between the thyroid gland and iodine has long been recognized. The discovery of considerable amounts of iodine within the gland, by Baumann, is but one of the important steps in the development of this conception. It is now well established that iodine is a constant constituent of the blood. Recent investigation is attempting to define more clearly this interrelationship in thyroid disease, particularly in regard to the iodine content of the blood, of the thyroid gland, and to a certain extent of the other tissues. One difficulty in the progress of this study has been the development of adequate micromethods for determining the small amounts of iodine normally present within the blood and tissues. An answer to this has been furnished within the past decade, particularly in the work of von Fellenberg. Much remains to be learned regarding the interrelationship between the thyroid, iodine metabolism, and goiter. As a consequence, it has seemed worth while to develop available methods for the determination of the small amounts of blood iodine and to apply them in more extensive metabolic studies. It is trite to state that the application of the newer methods is of value in the development of knowledge in such a field. We wish to present at this time the method with which Davis experimented for five months and which subsequently proved, in his hands, successful. Later we shall present results which we have obtained in applying this method to the study of the blood in the various diseases of the thyroid gland.

The principles upon which the method we have used is based were developed by Winkler[1] who made use of the fact, previously discovered by him,[2] that an iodide may readily be oxidized to an iodate.** In our work we have used bromine for this oxidation, according to the formula

$$I + 5\,Br + 3\,H_2O \rightarrow 5\,HBr + HIO_3.$$

The iodate thus formed readily reacts with an excess of hydriodic acid (KI in

*This work has been conducted under a grant from The Douglas Smith Foundation for Medical Research of The University of Chicago.

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Received for publication, Dec. 7, 1931.

**Owing to the significance of this contribution, commonly known as the Winkler principle, in the development of modern micromethods for the determination of iodine, it is of interest that A. and F. Dupré,[15] working in Heidelberg under Bunsen, had a similar method in mind. They showed, when iodine is gradually liberated from iodides by free chlorine water, that the extent to which the iodine may be shaken out by carbon disulphide or chloroform depends upon the amount of chlorine water added, but that if one adds a sufficient excess of chlorine water none of the iodine remains free to be dissolved in the carbon disulphide or chloroform. They considered that the inorganic iodide was converted into free iodine, then ICl and finally ICl_3, as the amount of chlorine was increased. This ICl_3, when later treated with excess KI then gave 6 parts of iodine for each part of ICl_3. Thus their ratio is the same as in the present method. Their interpretation of the reaction, however, is probably incorrect, since the formation of iodate is now well established. We are indebted to Prof. F. C. Koch for this information, and for other valuable suggestions.

acid solution), liberating six parts of iodine for each part present in the original substance to be analyzed, according to the formula

$$HIO_3 + 5\ HI \rightarrow 3\ H_2O + 3\ I_2.$$

Thus is developed a six-fold greater titration value than that which would be given by the iodine originally present in the sample. It can readily be seen that this is of major importance in the analysis of material, such as blood, in which the iodine content is so low that it is given in terms of *gamma*. One gamma equals 0.001 mg. In this region the blood iodine of nongoitrous clinic and hospital patients averages 12 gamma per cent.

Von Fellenberg,[3] Leitch and Henderson,[4] McClendon[10] and others have utilized this principle, working it into methods adapted for use in the analysis of material containing much organic matter and but little iodine. To von Fellenberg should go most of the credit for developing the method whereby the organic matter is eliminated without loss of iodine. Von Fellenberg's method has been widely utilized by Lunde and his associates and by Sturm.

The material to be analyzed, in this instance blood, is first ashed by heating with KOH, after which the iodine is extracted as KI. The extract is acidified and the resulting HI is oxidized to HIO_3 by boiling with bromine water. The HIO_3 is then allowed to react with an excess of KI (still in acid solution) and the iodine set free is titrated against N/500 sodium thiosulphate using starch solution as an indicator.

This titrimetric method, as employed by von Fellenberg,[3] Leitch and Henderson,[4] Veil and Sturm,[5] Lunde and Closs,[6] and others has been fully described. We are repeating it here as we have utilized it in order to give the benefit of our particular experience with the method and to point out certain of the numerous pitfalls which will be mentioned later under discussion of the method.

REAGENTS

Potassium hydroxide—40 per cent.—This reagent is made by dissolving 400 grams of KOH, "Kahlbaum," in 600 c.c. of distilled water and diluting to 1000 c.c.

Potassium nitrate—15 per cent.—One hundred and fifty grams of KNO_2, "Kahlbaum," is dissolved in distilled water and diluted to 1000 c.c.

Ethyl alcohol—90 per cent.—Redistilled.

Sulphuric acid—2N.—Add 5.6 c.c. of concentrated H_2SO_4 to 100 c.c. distilled water. This solution will keep indefinitely.

Potassium iodide—10 per cent.—Dissolve 5 grams in 50 c.c. of water. Make up fresh each time used. This solution need only be approximate.

Bromine water.—A saturated solution, made by shaking up 2 c.c. of bromine in 100 c.c. of distilled water. Owing to the possibility of some bromic acid formation, this should be prepared fresh every few days and kept in the dark.

Methyl orange.—0.05 per cent solution.

Potassium biniodate—N/10.—This, the real standard used, is made by dissolving 3.2496 grams of potassium biniodate (very accurately weighed) in water and diluting to one liter. Shaffer and Hartmann[13] give excellent directions for preparing a very high grade potassium biniodate.

Sodium thiosulphate—N/10.—Dissolve 25 grams of the pure crystalline salt

in recently boiled distilled water. Dilute to 1000 c.c. with recently boiled distilled water. Standardize this as follows: Measure off 25 c.c. of the N/10 potassium biniodate into a 250 c.c. Erlenmeyer flask. Add 100 c.c. distilled water, 1 gram KI and 5 c.c. of 10 per cent HCl. Titrate at once to a light yellow color with the sodium thiosulphate. Add 1 c.c. of the 1 per cent starch solution and titrate to a colorless end-point. Due to the large amounts of reagents used, this end-point is sharp and offers no difficulties. The thiosulphate solution is then diluted until it is within 0.2 per cent of N/10. Further accuracy is not needed. Acidity and microorganisms cause decomposition of the sodium thiosulphate. As a consequence it should be protected from atmospheric carbon dioxide and contamination, and kept in the dark; or it should be rendered slightly alkaline by adding sodium carbonate, as recommended by Mayr and Kerschbaum.[14] N/500 sodium thiosulphate is made fresh from this stock solution, each time as needed, by accurately diluting 2 c.c. to 100 c.c.

Starch solution—1 per cent.—Made by mixing 1 gram soluble starch with 100 c.c. of distilled water and boiling 1 minute.

PURITY OF REAGENTS

Kahlbaum's reagent grades of KOH and KNO_3 have been repeatedly tested for iodine content. Ten times the amount of these reagents used in the determination will give no trace of color with the starch solution when run as a "blank." The alcohol should be similarly tested and if found to be contaminated by iodine, should be redistilled from solid KOH.

To test the H_2SO_4 and bromine water, titrate 5 c.c. of the 2N acid with KOH, using methyl orange as an indicator. Bring the solution back to the acid side of the indicator with 1 drop of the acid. Add 5 c.c. of bromine water and boil until the volume of the solution is about 2 c.c. Add 5 drops of 10 per cent KI and 5 drops of 1 per cent starch solution. There should be no trace of color, as a blue color at this point would indicate the presence of iodine in the bromine. Iodine is a common impurity in bromine and the bromine should be tested carefully.

Ordinary C. P. sodium thiosulphate and potassium iodide are sufficiently pure.

PROCEDURE

In drawing blood from the patient, great care must be taken to avoid contamination. A chemically clean but not necessarily sterile syringe is used. The blood is taken from one of the cubital veins after carefully cleansing the skin with pure alcohol. *No iodine is used and there should be no iodine on the clean-up tray.* At least 22 c.c. of blood should be drawn and divided immediately between two one-ounce bottles, each containing 5 mg. of sodium oxalate or sodium citrate. Either oxalate or citrate may be used. The bottles should be shaken thoroughly by rapid rotation.

By means of an outvolume pipette, measure accurately 10 c.c. of whole blood into a 50 c.c. nickel crucible. Add 2.5 c.c. of 40 per cent KOH solution and stir with a fine-pointed glass rod. Rub the adherent mixture off the tip of the rod against the side of the crucible. It is impractical to rinse off the rod. Put the crucible containing the mixture into an oven kept at 120° C. and allow it to remain there for at least twelve hours. This dries the blood so

that it can be heated to a char without excessive bubbling. *The oven used in this and succeeding steps should never be used for any other purpose than for iodine determinations.* After drying, the blood and KOH mixture is heated to a char. This heating is done by placing the nickel crucible in a large iron crucible about 8 cm. in diameter. In the bottom of the iron crucible is a layer of sand about 5 mm. deep. The iron crucible is then heated over a Bunsen burner in a hood. In this and in succeeding heatings the nickel crucible should never be heated to a glow. When the mixture has become a dry char, the two crucibles are transferred to a Chaddock burner and the heating continued. At ten-minute intervals, the nickel crucible is taken out and allowed to cool. The contents are moistened with 15 per cent KNO_3 solution and the nickel crucible is returned to the iron one. This procedure is continued until all the carbon particles are oxidized and the content is a fused reddish mass.

Distilled water is then added to the ash in 3 c.c. portions. Each portion, after standing a few minutes, is filtered through a No. 40 Whatman filter paper into a second nickel crucible. After the ash has been all dissolved, the crucible is rinsed with several 3 c.c. portions of water and the rinsings are also poured through the filter. Rinse the filter paper once and discard it, since repeated determinations have failed to detect the presence of iodine. The crucible containing the filtrate is now transferred to the 120° oven and evaporated to dryness on a grid in the upper part of the oven.

Enough distilled water is added to the residue to dissolve it on heating. This requires 1 to 1.5 c.c. Return to the oven and evaporate until the solution begins to solidify. Add 3 c.c. of the 90 per cent alcohol while still hot. Cool and rub the KOH mixture to a paste. This is best done with a nickel or iron rod about 3 mm. in diameter with a chisel-shaped end. *It is extremely important that there should be just the right amount of water left in the KOH mixture when the alcohol is added.* Recognition of this point is a matter of experience. The best criterion lies in the amount of 2N sulphuric acid required to titrate the alcoholic extract. This should be between 10 and 20 drops. When more acid must be used, the K_2SO_4 interferes with the final titration; when less is used, the solution is not buffered sufficiently and the acidity developed is so high that the KI added is oxidized by atmospheric oxygen.

The alcohol is decanted off and filtered through a No. 40 Whatman filter into a 50 c.c. Erlenmeyer flask. The paste is then extracted by three more 3 c.c. portions of 90 per cent alcohol. Each of these portions should be allowed to drain through the filter before the succeeding one is decanted into the filter. After the last extraction, the paste is transferred to the filter and allowed to drain for thirty minutes. The funnel is rinsed once after the filter has been removed. The Erlenmeyer flasks are then transferred to the oven and the filtrate evaporated to dryness.

The flasks containing the evaporated filtrate are removed from the oven, cooled, and the film of salts is dissolved in 3 to 5 c.c. of distilled water. Two drops of the 0.05 per cent methyl orange solution are added and the solution is titrated to the methyl orange end-point with 2N H_2SO_4, one drop of acid being added in excess. This titration is a delicate one. The red color of the methyl orange on reaching the acid side of the end-point is bleached almost

immediately if much HIO_3 is present so the end-point must be approached cautiously. Bromine water is added drop by drop until the solution is distinctly yellow. Two small pieces of pumice stone are dropped into each flask and the solutions are boiled until the excess bromine is driven off and the volume of the solutions is from 0.5 to 1 c.c. The flasks are cooled and from 2 to 5 drops of 10 per cent KI solution and 2 drops of starch solution are added to each flask. First add 2 drops of KI; if the solution turns yellow, add 3 more.

The solutions are now titrated with freshly prepared N/500 sodium thiosulphate solution. For this titration is used a 0.2 c.c. microburette, graduated in thousandths of a cubic centimeter, and with an attached reservoir. The pipette tip of this burette is drawn out to a fine point. With this pipette three cubic millimeters (0.003 c.c.) can be accurately delivered by means of the burette stopcock. The minute droplet so delivered is added by touching the tip to the flask containing the solution which is being titrated. As the end-point is approached, the thiosulphate is added in 0.003 c.c. increments. When the point is reached where it is doubtful as to whether any color remains, we usually add a full drop. Then, if there is any further color change, we add 0.002 to 0.005 c.c. to the titration readings according to the amount of color change.

CALCULATION

The calculation is based on the formula:

Thiosulphate used $\times \frac{\text{iodine equivalent of thiosulphate}}{6}$ N/500 iodine contains $\frac{127}{500}$ grams per liter. Hence, 1 c.c. of N/500 sodium thiosulphate is equivalent to $\frac{127}{500 \times 1000}$ or 254 gamma of iodine. Consequently $\frac{254}{6}$ or 42.3 gamma is the iodine equivalent, *in the original sample*, of each cubic centimeter of thiosulphate. Since 10 c.c. blood samples are used, the gamma per cent of the blood iodine is 423 times the number of cubic centimeters of sodium thiosulphate used in the final titration.

DISCUSSION

This method for the quantitative determination of the blood iodine is by no means an easy one. We feel that its success in the ordinary clinical laboratory would be doubtful. It requires experience and practice to obtain satisfactory results. It is best carried out in a laboratory used solely for that purpose and one in which no tincture of iodine or other volatile form of iodine is ever allowed to be present. Such reagents used in the procedure as contain iodine should be handled with extreme care to prevent possible iodine contamination of the blood samples.

After five months of thorough experimentation, this method was applied to samples of starch solution to which had been added known quantities of iodine. Davis was able to differentiate definitely between samples which contained 0.8 gamma, 8 gamma per cent, and those which contained 1.0 gamma, 10 gamma per cent. After working with about 500 samples during the past eighteen months, Davis can make duplicate determinations on blood samples containing 0.8 to 1.2 gamma with a variation between duplicates of about 0.05 gamma, or an error of from 6 to 8 per cent. In succeeding papers we shall

report studies on the blood iodine in normal individuals and in patients with diseases of the thyroid gland.

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THE EFFECT OF SODIUM THIOCYANATE ON THE ACTION OF ANESTHETIC AND NARCOTIC DRUGS*

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Wilder D. Bancroft and J. E. Rutzler, Jr.[1] have presented several experiments in vivo which they believe confirm Claude Bernard's theory of the phenomenon of anesthesia, which is: Anesthesia results when the colloids of the sensory nerve cells are reversibly coagulated (the coagulating agent is the anesthetic) and if the anesthetic is displaced by a substance that causes weaker flocculation, the biocolloids are peptized by the electrolytes within the cells and there is a return of irritability and consciousness. These two workers chose sodium thiocyanate as the peptizing agent in most of their experiments. They believe that the sensory nerve colloids are albumen-like and should adsorb the thiocyanate ion strongly and be easily peptized by the cell electrolytes.

In their paper they reported but one experiment each with the following anesthetic drugs: ether, sodium amytal, morphine, alcohol, and sodium nembutal, and also one experiment each with anaphylaxis, histamine, and strychnine poisoning.

The experimental technic used in their report does not seem adequate for

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Received for publication, February 18, 1932.

conclusive results. In both the ether and amytal experiments the rabbits were chosen so young (weight 600 gm.) that they could not be deemed normal mature animals.

In the experiments in which the sodium thiocyanate was used as an antagonist for ether, the control animal, which received ether alone, a few hours later underwent the sodium thiocyanate and ether combination. Certainly the second time this rabbit could not be considered normal and probably required much less ether to depress it so that its recovery was much more rapid.

Two young rabbits were used in the experiment with sodium thiocyanate and sodium amytal. Bancroft and Rutzler stated in their publication that the hind legs of the experimental animal were not normal during the recovery period while the control animal was normal. Had they performed more than one such experiment they would have noticed that every animal which receives the full dose of sodium thiocyanate shows this peculiar inability to control the hind legs, and as the dose is increased, the paralytic effect increases until all the legs are paralyzed and death often follows within twenty-four hours. We found this paralytic effect in our animals which received sodium thiocyanate and morphine in the same dosages as employed by Bancroft and Rutzler. Upon reducing the dosage of sodium thiocyanate only the effect of the morphine was evidenced.

The fact that only one experiment with each anesthetic was performed by these workers limits the value of their results. In experiments where two animals were used, the one receiving the thiocyanate might easily have been a rabbit which threw off the anesthetic more rapidly than the control, since there is a great variation in reaction to anesthetics by different animals of the same species as well as the same animal. However, in each of their experiments the results observed seemed to confirm their theory that sodium thiocyanate would increase the rate of recovery from anesthesia produced by these agents as well as from anaphylactic shock and strychnine poisoning. They conclude that:

1. "Sodium thiocyanate relieves to a greater or lesser extent the effect of these drugs."

2. The action of the sodium thiocyanate is due to the thiocyanate ion which peptizes the colloids that are coagulated by the above mentioned drugs.

3. "Sodium thiocyanate apparently peptizes the colloids of the respiratory center." This theory is based on the fact that with amytal the respiratory recovery was 130 per cent faster in the experimental animal than in the control animal.

4. "The use of too much sodium thiocyanate in the presence of a strong narcotic may cause sodium poisoning."

5. Sodium thiocyanate may relieve drug addiction by the peptizing of the nerve colloids of the brain.

6. The thiocyanate ion acts upon the protein of the central nervous system, including the brain, spinal cord and sympathetic nerves.

7. "No evidence has been found or adducted that the lipoids play a dominant rôle in the phenomena studied."

8. "Claude Bernard's theory of anesthesia as developed by Bancroft and Richter has been found to be inescapable."

In subsequent papers Bancroft and his associates[1] have discussed the application of this theory to the treatment of insanity, alcoholism and drug addiction.

In this laboratory an attempt has been made to follow with adequate controls the exact experiments described by Bancroft and Rutzler, using sodium thiocyanate with ether, morphine, and sodium amytal, in order to determine

whether such results as they observed in a very limited number of experiments are constant phenomena justifying important conclusions.

EXPERIMENTAL STUDY

I. Ether

Experiments using ether and sodium thiocyanate were performed on rabbits. The type experiment consisted essentially of placing a rabbit upon a rabbit board with its back down and securely tied. A weighed amount of sterile gauze was placed in a small ether mask and 10 c.c. of Squibb's pure ether was poured upon it. The mask was placed firmly over the rabbit's nose. The animal usually struggled violently at first, due to displeasure produced by the odor of the ether. Later they struggled because they were entering the excitement stage. The administration of the anesthetic was continued for ten seconds after the lid reflex had disappeared. This is a very deep state of anesthesia. Then the ether mask was removed and the animal freed. Records were taken of the time of the return of the lid reflex, of the time when the animal sat up, and finally the complete return to normal. Return to normal was judged by the ability of the animal to hop without staggering.

The same animal was then allowed several hours or even a day to rest. The experiment was then repeated exactly with the exception that during the last minute, while the ether was being given, sodium thiocyanate solution was injected into the marginal vein of the ear. (The amount of sodium thiocyanate was 0.00015 gm. per gram of body weight which was the same as used in the Bancroft-Rutzler experiment. A 10 per cent solution was used.) The records were then compared with those of the controls.

On July 15 three healthy, young, snuffle-free rabbits were selected and a control run on each as described above. The results are as follows:

Rabbit A 206.—Weight 1007 gm. Temp. of room 82° F. (1) Lost lid reflex in three minutes, twelve seconds. (2) Removed ether mask in three minutes and twenty-two seconds. (3) Lid reflex returned in three minutes and forty-five seconds. (4) Considered normal in twenty-six minutes and forty-three seconds.

In this case twenty-three seconds elapsed between the removal of the mask and the return of the lid reflex. The animal was normal in twenty-three minutes and twenty-one seconds.

Rabbit A 164.—Weight 1545 gm. (1) Lost lid reflex in two minutes and fifty seconds. (2) Removed ether mask in three minutes. (3) Return of lid reflex in three minutes and twenty-three seconds. (4) Considered normal in twenty-four minutes.

Lid reflex returned in twenty-three seconds and the animal was considered normal in twenty-one minutes after the ether mask was removed.

Rabbit A 173.—Weight 1440 gm. (1) Lost lid reflex in two minutes and fifty-three seconds. (2) Removed mask in three minutes and three seconds. (3) Returned lid reflex in three minutes and twenty-seven seconds. (4) Considered normal in twenty-five minutes.

Lid reflex returned in twenty-four seconds and the animal was considered normal in twenty-one minutes and fifty-seven seconds after the ether mask was removed.

These controls show that the time of the return of the lid reflex was nearly constant. The time was twenty-three, twenty-three, and twenty-four seconds respectively. The time for the return to normal varied but slightly, as follows:

Rabbit A 206, twenty-three minutes and twenty-one seconds; A 164, twenty-one minutes; A 173, twenty-one minutes and fifty-seven seconds.

This shows but little individual variation.

Six days later (this seemed to be time enough for the rabbits to be considered normal) July 21, these same three rabbits were used in the identical manner with ether plus sodium thio-

cyanate using a 10 per cent solution and giving 0.00015 gm. per gram body weight. This thiocyanate solution was administered intravenously into the ear vein during the last minute of anesthesia. The results are as follows:

Rabbit A 206.—Weight 1007 gm. received 1.5 c.c. of 10 per cent solution of sodium thiocyanate. (1) Lost lid reflex in four minutes and thirty seconds. (2) Removed ether mask in four minutes and forty seconds. (3) Lid reflex returned in four minutes and fifty-seven seconds. (4) Considered normal in twelve minutes and five seconds.

The lid reflex in this case returned in seventeen seconds and the return to normal was in seven minutes and twenty-five seconds. This animal died two days after the experiment.

Rabbit A 164.—Weight 1545 gm. received 2.3 c.c. of 10 per cent solution of sodium thiocyanate. (1) Lost lid reflex in three minutes and eighteen seconds. (2) Removed ether mask in three minutes and twenty-eight seconds. (3) Return of lid reflex in three minutes and thirty-nine seconds. (4) Considered normal in eleven minutes and five seconds.

The lid reflex returned in eleven seconds and the rabbit was normal in seven minutes and thirty-seven seconds.

Rabbit A 173.—Weight 1440 gm. received 2.16 c.c. of 10 per cent solution of sodium thiocyanate. (1) Lost lid reflex in six minutes and eighteen seconds. (2) Removed ether mask in six minutes and twenty-eight seconds. (3) Return of lid reflex in six minutes and forty-five seconds. (4) Considered normal in fourteen minutes and two seconds.

The lid reflex returned in seventeen seconds and the rabbit was normal in seven minutes and thirty-four seconds after the ether mask was removed. It died three days later.

The following chart summarizes:

Rabbit	Return of lid reflex (After mask is removed)	Return to normal
	Ether	
A 206	23 sec.	23 min. 21 sec.
A 164	23 sec.	21 min. 0 sec.
A 173	24 sec.	21 min. 57 sec.
	Ether plus Sodium Thiocyanate	
A 206	17 sec.	7 min. 25 sec.
A 164	11 sec.	7 min. 37 sec.
A 173	17 sec.	7 min. 34 sec.

This set of three rabbits, in which the control was run first, certainly gave results which point toward a more rapid return of the lid reflex and the return to normal with the use of sodium thiocyanate than without it. The results show little variation.

A reversal of the order of the experiment was then made, running the experiment first, followed by the controls. The same procedure was followed, using three new rabbits. One day was allowed for recovery between the experiment and the control.

The following chart summarizes:

Rabbit	Return of lid reflex (After ether mask is removed)	Return to normal
	Ether plus Sodium Thiocyanate—July 20	
A 119	35 sec.	23 min. 50 sec.
A 123*	25 sec.	10 min. 8 sec.
A 116*	32 sec.	15 min. 44 sec.
	Ether—July 21	
A 119	22 sec.	17 min. 20 sec.
A 123	22 sec.	17 min. 0 sec.
A 116	20 sec.	19 min. 44 sec.

*Rabbits A 123 and A 116 died four and five days later respectively.

In this set of experiments the reversal of the order gave results which are contradictory to those of the first set as far as the return of the lid reflex is concerned. The return to normal with the use of thiocyanate in numbers A 123 and A 116 was more rapid than with the controls, as Bancroft and Rutzler stated, but much slower with A 119, which is just opposite to their theory. The recovery of A 123 with thiocyanate was only about half as rapid as in the preceding group, while all the lid reflexes required a much longer time in this group. We have, then, a decided variation in rate of recovery between the two groups. Bancroft and Rutzler did not report a reversal of the order of the experiment and therefore were not aware of the fact that the animals do not show the same rate of recovery when the experiment precedes the control as they do when it follows the control.

It was noticed soon after the injections of the sodium thiocyanate solution that the intestines were in exceedingly active motion. This was not noticed in the control animals.

Of the six animals used in these two groups of experiments four developed intestinal disturbances with diarrhea and died within two to five days after the injection of the thiocyanate. During this stage they refused food, lost weight rapidly, became quite weak and died with slight convulsive jerks of their legs and body and marked extension of the hind legs. Autopsy revealed no recognizable lesions or changes in the organs. These toxic symptoms were not observed by Bancroft and Rutzler.

An experiment was then run upon one rabbit to determine if there were any individual variations with the use of ether alone which might account for the fact that one rabbit contradicted their results.

Rabbit A 165 was run on July 13 with ether only. (1) Lost lid reflex in ten minutes and eight seconds. (2) Removed ether mask ten minutes and twenty-eight seconds. (3) Return of lid reflex twelve minutes and eight seconds. (4) Considered normal in twenty-five minutes and ten seconds.

Two days later, July 15, this same rabbit was run with ether again. (1) Lost lid reflex in three minutes and twenty-five seconds. (2) Removed ether mask in three minutes and forty-five seconds. (3) Reflex of lid returned in four minutes and twenty seconds. (4) Considered normal in twenty-two minutes and fifteen seconds.

In the first case the lid reflex returned in one minute and forty seconds after stopping the anesthetic and in the second case thirty-five seconds. It took much longer in the first experiment for the rabbit to lose consciousness. Perhaps it was becoming accustomed to ether by the time of the second experiment. It will be noticed in the first experiment that the return to normal was fourteen minutes and forty-two seconds while in the later experiment it required eighteen minutes and thirty seconds; although the return of the lid reflex was much faster than in the first experiment. Evidently there is some individual factor to be considered in these experiments and the variations obtained before cannot be attributed to thiocyanate alone.

These results called for an experiment with a fresh rabbit using ether and sodium thiocyanate together and repeating in a few days, to see if there is any individual variation with thiocyanate.

Rabbit A 168, weighing 1640 gm. was selected July 29. It received the usual

10 c.c. of ether, followed by 2.46 c.c. of 10 per cent solution of sodium thiocyanate in the marginal vein of the ear during the last minute of anesthesia.

The lid reflex returned in twenty-six seconds and the animal was normal in nine minutes and twenty-one seconds. Two days later the same experiment was run on the same rabbit. This time the lid reflex returned in twenty-three seconds and the animal was normal in five minutes and one second. This shows a marked increase in the rate of recovery during the second experiment, although the same technic and the same dosages were used. If the thiocyanate had not been used in the first of these tests, we could easily have believed that the thiocyanate had caused the more rapid recovery in the second test. This indicates that the increased rate of recovery with the use of thiocyanate in the earlier experiments cannot be definitely attributed to the use of that drug.

Rabbits A 73 and A 166 were run in like manner at the same time, using ether and 10 per cent solution of sodium thiocyanate.

Rabbit A 73 had a return of lid reflex in twenty seconds and was normal in five minutes and forty-two seconds. Two days later in the second run the lid reflex returned in eleven seconds and the animal was normal in five minutes and forty-six seconds. Here the return to normal was almost an exact check.

Rabbit A 166 had a return of the lid reflex in twenty seconds and was normal in five minutes and ten seconds, and in the second run two days later the lid reflex returned in twenty-seven seconds and the animal was normal in four minutes and twelve seconds. We can conclude that rabbits A 168 and A 167 show individual variations while A 73 is quite constant.

This is another experiment which shows that rabbits do not always react in the same way to sodium thiocyanate when it is used as an antagonist for anesthetic drugs.

Rabbit A 166 died six days later and rabbit A 168 died ten days later with no gross nervous lesions, but both did show loss of weight.

At this point it became evident that conclusive results could not be obtained by this procedure since marked deviations continued to occur in experiments that were identically the same. However, experimentation was continued with the intention of giving the problem a fair trial.

Two fresh rabbits were subjected to the usual ether-sodium thiocyanate experiment and followed eleven days later by the control. Rabbits A 143, weight 1650 gm. and A 95, weight 1730 gm. received respectively 2.47 c.c. and 2.59 c.c. of 10 per cent sodium thiocyanate solution during the last minute of anesthesia. Rabbit A 145 returned to normal in eight minutes and fifty-seven seconds and A 95 was normal in five minutes and thirty-nine seconds, although both experiments were conducted in identically the same manner. Eleven days later these two rabbits underwent the control experiment using ether alone. Rabbit A 143 returned to normal in six minutes, or two and fifty-seven seconds quicker with ether alone than with thiocyanate.

However Rabbit A 95 required ten minutes and fifty-four seconds to return to normal with ether or nearly twice as long as with thiocyanate. This experiment is more in accord with the results obtained by Bancroft and Rutzler and might have led them to believe that they had discovered an antagonist for anes-

thesia. Nevertheless this increased rate of recovery with thiocyanate may not be due to the effect of this drug but simply another example of individual variation such as has been seen before.

The fact that this one experiment was similar to the reports of the Cornell workers led to a group experiment on four more rabbits. These rabbits were selected so that they were of the same sex and very near the same weight. Two were run in the usual way as controls with ether alone and two received the ether-thiocyanate treatment.

The chart shows the result of these four experiments.

Rabbit	Weight	Return of lid reflex	Normal
		Morning: Ether	
A 185	1720 gm.	21 seconds	14 min. 42 sec.
A 187	1880 gm.	15 seconds	17 min. 17 sec.
		Afternoon: Ether and Sodium Thiocyanate	
A 185	1720 gm.	28 seconds	16 min. 22 sec.
A 187	1880 gm.	59 seconds	10 min. 48 sec.
		Morning: Ether and Sodium Thiocyanate	
A 31	1880 gm.	14 seconds	10 min. 17 sec.
A 39	1770 gm.	59 seconds	10 min. 2 sec.
		Afternoon: Ether	
A 31	1880 gm.	21 seconds	11 min. 0 sec.
A 39	1770 gm.	38 seconds	8 min. 27 sec.

Although this experiment was run the same day, the experiments were reversed so that the two rabbits receiving the ether alone in the morning received ether and sodium thiocyanate in the afternoon, and those that received sodium thiocyanate in the morning received ether in the afternoon. The results showed that rabbit A 187 recovered more quickly to normal under the influence of sodium thiocyanate than when it had ether only. However the rabbits A 185 and A 39 took longer to recover with the use of sodium thiocyanate. Rabbit A 31 returned to normal in almost the same time with or without thiocyanate.

Seven out of seventeen rabbits in these experiments which received the regular dose of sodium thiocyanate died within two to ten days, showing only weakness, loss of weight and diarrhea. Autopsy revealed no gross lesions.

It was thought that the sodium ion might have some effect on these results, and so three fresh rabbits were selected and anesthetized with ether as before, and an equimolar solution of sodium chloride as that of the sodium thiocyanate was injected intravenously into the marginal vein of the ear of anesthetized animals as in the earlier experiments. The results in two rabbits show that the sodium chloride tends to cause deeper and slightly quicker anesthesia with slower return to normal and must, according to the theory of Bancroft and Rutzler, act as a colloidal protein coagulant for the central nervous colloids, causing deeper anesthesia than with ether alone. In the case of rabbit A 16 the return to normal with sodium chloride was almost twice as rapid as with ether alone. Since this occurred several times with the sodium thiocyanate we cannot say that the

more rapid recovery in the latter cases is due to the thiocyanate ion since it also occurs with sodium chloride.

Sodium tartrate was used on two other rabbits instead of thiocyanate. These rabbits reacted much as did the last rabbit which received the sodium chloride. They returned to normal twice as rapidly with sodium tartrate as with ether alone. It may be that in these three cases the sodium ion depressed the animals so quickly that they passed into deep anesthesia with less ether than is ordinarily required. When the tissues lost the sodium ion the animals were able to recover much more quickly since they did not have so much ether in their systems.

II. SODIUM AMYTAL

Since ether is so easily eliminated from the body, Bancroft and Rutzler decided to give sodium thiocyanate a more difficult task. Consequently they selected sodium amytal.

Following the method of Clowes these workers slowly injected 5 mg. of sodium amytal in water solution per hundred grams of body weight into the marginal vein of the ear of a young rabbit. This rabbit was used as a control. The animal was limp before the injection was complete. The respiration was only 14 per minute after forty-two minutes and there was no response to a needle prick. After two hours and twenty-eight minutes the rabbit began to stir and one minute later was definitely conscious. It was unable to sit up after three hours and fourteen minutes. The rabbit was up and about four hours and fourteen minutes after the induction of the anesthetic.

At the same time a litter-mate was anesthetized in an identical manner with sodium amytal. Four minutes after the amytal was given, 1 c.c. of 10 per cent solution of sodium thiocyanate for each 600 gm. of body weight was injected intravenously into the marginal vein of the ear. The rabbit raised its head and worked its legs before the injection was completed and the lid reflex returned fifteen minutes later. When twenty-five minutes had elapsed, 0.5 c.c. more of the thiocyanate solution was injected subcutaneously. The rabbit distinctly felt the needle. The animal then arose of its own accord and the respiration was 32 per minute after thirty-three minutes of narcosis, or 130 per cent faster than the control. Three more small injections were made from time to time which totaled 1.2 c.c. more, all subcutaneously. After the next to the last of these the animal crawled but its hind legs were not completely normal. The last injection of thiocyanate caused the legs to become normal and the rabbit hopped about although still intoxicated. After two hours and ten minutes of narcosis the animal was able to hop around after first crawling a short distance.

The rate of respiration was twice as fast in the last animal as in the control, due to the depressant effect of the anesthetic in the control animal.

Bancroft and Rutzler believe this fact evidences the displacement and peptizing action of the thiocyanate upon the respiratory center. They also state that the slight awkardness of the hind legs is due to intoxication which resulted when the thiocyanate was struggling to overcome the amytal.

The above experiments have been repeated on a larger scale, and the following abstracts of the protocols give the results obtained in nine experiments in

testing sodium thiocyanate as an antagonist for sodium amytal, the procedure employed in the first four and the sixth and seventh amytal experiments being an exact repetition of that used by Bancroft and Rutzler.

Experiment I.—

Control (sodium amytal alone)	*Experimental* (sodium amytal and thiocyanate)
Rabbit AO: weight 1830 gm.	*Rabbit A 238:* weight 1970 gm.
1. Lid reflex did not disappear completely after injection of sodium amytal.	1. The lid reflex was weak after the injection.
2. Rabbit showed no feeling to needle prick on abdomen after thirty-three minutes.	2. Showed feeling to needle prick after twenty-nine minutes.
3. Respiration was 32 per minute after thirty-five minutes.	3. Respiration was 34 per minute after thirty-five minutes.
4. Animal able to sit up and it hopped feebly after four hours and forty minutes.	4. After two hours and forty minutes the animal was still unable to rise and showed signs of spasmodic contractions of the hind legs.
5. The hind legs are normal.	5. After four hours and forty-five minutes the animal was still unable to rise and showed signs of spasmodic contractions of the hind legs.
	6. The animal died during the night never having recovered.

Experiment II.—The control animal recovered to normal after two hours and twenty minutes and at no time showed convulsive contractions. The experimental animal was nearly normal after four hours and thirty minutes. Its head was rotated to the right. It traveled with a running gait and often fell to the left.

Experiment III.—The results of this experiment are very similar to experiment II. The control rabbit was normal after four hours and four minutes while the animal receiving the thiocyanate was nearing normal after six hours and ten minutes. Its hind legs were stiff and it was unable to control them.

Experiment IV.—The results are much the same except that the animal receiving the thiocyanate recovered fifty-two minutes sooner than the control but it showed a slight stagger as it hopped and was found dead three days later.

Since two of the four rabbits which received the thiocyanate and amytal died, and all four showed marked toxic effects, I decided to reduce the dose of sodium amytal one-half and see if the sodium thiocyanate might overcome weaker doses without producing these disturbing effects.

Experiment V.—One-half the previous dose of sodium amytal was used. The control animal was quite normal after fifty-nine minutes. The experimental animal was normal in one hour and twenty-two minutes. Its hind legs were stiff and awkward.

Since there was little improvement in the results with half doses of amytal, the original dosage was employed again.

Experiment VI.—The control animal was normal in two hours and fifty minutes. The experimental animal was still depressed after three hours and twenty minutes—the next day it was normal but the second day developed severe diarrhea and lost weight.

Experiment VII.—The control animal was normal after three hours and twenty-four minutes, while the experimental animal was unable to sit up after five hours. It died during the night.

Experiment VIII.—In this experiment sodium citrate was used in place of sodium thiocyanate as an antagonist for sodium amytal. The control animal was normal in three hours

and thirty minutes. The experimental animal died following the intravenous injection of the sodium citrate. Sodium citrate probably caused overalkalinity of the blood with depression of the respiratory center.

Experiment IX.—In this case the dosage of sodium thiocyanate was reduced one-half and the sodium amytal remained the same as before. Both rabbits recovered in three hours and thirty-six minutes. This was probably due to the fact that the sodium thiocyanate was too dilute for its action to be evidenced. The effects were those due to the sodium amytal alone since both animals reacted in the same manner and the experimental animal showed no toxic effects.

Throughout the eight experiments with sodium thiocyanate (disregarding the one in which sodium citrate was used), the response to the needle prick was identical in each set, so that no appreciable difference in response could be demonstrated between the reaction of the control and that of the experimental animal. The respiration in none of the controls was as low as that recorded by Bancroft and Rutzler, which was 14 per minute after forty-two minutes of narcosis. The lowest respiration in our animals was twenty-four per minute after forty-two minutes of narcosis. Not one of the experimental animals arose of its own accord after the injection of the sodium thiocyanate, as Bancroft and Rutzler report occurred after the first injection and after each successive injection. In no case did the respiration of the experimental animal exceed that of the control more than two or three per minute. In three sets of experiments the respiration of the experimental animal was considerably slower than that of the control, which makes one suspect that the sodium thiocyanate depresses the respiratory center rather than stimulates it.

It is interesting to note that our controls recovered to normal on the average of four hours and eight minutes of anesthesia. This almost checks with that of the one experiment of Bancroft and Rutzler which was four hours and fourteen minutes, but our animals never completely lost their lid reflexes nor had as low respiratory rates. Our experimental animals, with the exception of the fourth set, required from one-third to twice as long to return to normal as did the controls, and three out of seven which received the injection of the 10 per cent solution of sodium thiocyanate died within three days, while none of the controls died. Those which did not die, as well as those which did die, showed severe nervous symptoms which resulted in the inability to control properly the movements of the rear extremities. In several cases the thiocyanate caused inability to move any of the legs or head in coordinate motion and the animals were unable to propel themselves even when conscious.

William Goldring and Herbert Chasis[2] who administered thiocyanate to hypertension patients, found that 17 per cent developed toxic manifestations with hallucinations of sight, convulsive movements of the extremities, coma and even death in two cases. These toxic effects were unrelated to the amount of thiocyanate administered and in some patients there was found to be little or no margin of safety between the toxic and therapeutically effective dose of thiocyanate.

III. MORPHINE

The last group of experiments consisted in testing the power of sodium thiocyanate as an antagonist against morphine. The dose of morphine sulphate

was 0.052 mg. per gram of body weight, this being the same as that used by Bancroft and Rutzler.

These workers reported one control animal compared with an experimental animal, and their results are briefly as follows:

The control rabbit, weighing 2300 gm., received 0.12 gm. of water solution of morphine subcutaneously. The animal was in deep narcosis after three hours and eight minutes at which time its respiration was 12 per minute. After five hours the respiration was only 18 per minute and the rabbit was still well under the influence of morphine.

At the start of the control experiment a companion rabbit was likewise narcotized. This animal was asleep in ten minutes. Five minutes later 3.8 c.c. of 10 per cent solution of sodium thiocyanate was injected intravenously, after which the rabbit was able to sit up although it was in a stupor. Later the rabbit appeared to be losing strength and 3 c.c. more of the thiocyanate solution were injected. This brought the strength back quickly and the respiration was 22 per minute after two hours and thirty-five minutes as compared to twelve of the control. After four hours the respiratory rate was thirty-eight per minute. Throughout the experiment this animal was on the border line of consciousness.

In my experiment four young rabbits were selected and subjected to the same experimental procedure as that used by Bancroft and Rutzler. Two were run as controls and received only morphine (0.052 mg. with water solution per gram body weight) while the other two received morphine followed by intravenous injections of sodium thiocyanate. The first pair consisted of rabbit A 234 which was run as a control and given morphine only, and rabbit A 363 which was given morphine plus sodium thiocyanate.

Rabbit A 234: weight 1950 gm. (1) After one hour and eleven minutes the respiration was 10 per minute. (2) After two hours and twenty-two minutes the respiration was 16 per minute. (3) After four hours and thirty minutes the animal turned over on its abdomen when placed on its side. (4) After seven hours it was rapidly nearing normal and able to hop about slowly. (5) The following morning it was normal and able to eat.

Rabbit A 363: weight 2010 gm. received 3 c.c. NaCNS after fifteen minutes; 3 c.c. NaCNS after one hour and four minutes; and 1 c.c. after five hours and three minutes. (1) After one hour and ten minutes the respiration was 12 per minute. (2) After two hours and twenty-four minutes the respiration was 17 per minute. (3) After four hours and thirty minutes it tried to turn on its abdomen when turned on its side but was unable to do so. Instead its hind legs extended backwards spasmodically and seemed to be out of control and somewhat paralyzed. (4) After seven hours the rabbit was still unable to move or sit up. The hind legs were still extended and its breathing was forced. (5) The following morning it was still unable to get up. Its hind legs remained extended and appeared to be completely paralyzed. It lay as if it were dead except for its breathing and its head was rotated 60 degrees to the right. When it was picked up it made slow, forced locomotor motions with all its feet. It had diarrhea. (6) The second morning it was found dead.

The second pair consisted of rabbit A 119 which was the control and rabbit A 123 which was the experimental animal.

Rabbit A 119: weight 1920 gm. (1) After one hour and twelve minutes it was sleeping quietly. (2) After two hours the respiration was 15 per minute. (3) After four hours the respiration was 16 per minute. (4) After five hours the respiration was 17 per minute, and the rabbit was nearing normal. (5) The next morning the rabbit was normal and lively.

Rabbit A 123: weight 2070 gm. received 3 c.c. of NaCNS after fifteen minutes and 2.4 c.c. of NaCNS after one hour and twelve minutes. (1) After one hour and twenty minutes

or eight minutes after the second injection of 2.5 c.c. of sodium thiocyanate which was injected intravenously, the rabbit extended its hind legs backward and became very sensitive to slight shock or noise. (2) After two hours the respiration was 15 per minute and the slightest touch caused rigid contractions which resembled strychnine contractions. (3) After four hours the respiration was 24 per minute. (4) After five hours the respiration was 20 per minute. (5) The next morning the rabbit was free from morphine symptoms but its head was rotated 30 degrees to the left and it hopped in a circle to the right. A slight tap or jar caused the animal to jump and jerk spasmodically. (6) The animal died during the following night.

It will be noticed that in the preceeding pair the experimental rabbit received a total of 7 c.c. of 10 per cent solution of sodium thiocyanate by the intravenous route. It weighed 2010 gm. In this last pair the experimental rabbit received only 5.4 c.c. by the same route although it weighed 2070 gm. This reduction however had no effect in the fatality of the dose as the animal was also found dead the second morning.

Since both of these animals died shortly after the experiment, it was decided that an animal should be subjected to the same dose of thiocyanate without the morphine in order to determine whether or not the effects of thiocyanate alone would cause death or whether the animal died as a result of the combined drugs. Rabbit A 73, weighing 2080 gm. was given enough ether to relax the muscles and received the identical dosages injected at the same interval which were given to A 363 and totaling 7 c.c. After the first injection the animal sat up when the ether mask was removed and hopped across the room as if it were normal. The second injection showed little effect but after the last was completed the rabbit was unable to hop normally. Its hind legs began to show signs of dragging and it bumped into objects in its path. Suddenly its front legs gave way and it sprawled on the floor unable to support its own weight. The fore legs remained extended to the sides as if the pectoral muscles had failed and the head and chest rested on the floor. The animal died during the night. It showed signs of diarrhea as did almost every rabbit which received either large or small doses of sodium thiocyanate throughout all of our experiments.

One more experiment was performed using morphine and thiocyanate but further decreasing the amount of the thiocyanate injected.

Rabbit A 95: weight 1900 gm. Control animal. (1) Received 0.098 gm. morphine. (2) After thirty-two minutes of narcosis the respiration was 60 per minute. (3) After one hour and twenty minutes the respiration was 67 per minute. (4) After three hours and twenty minutes the animal was still dull and in a stupor. (5) The next morning the rabbit showed complete recovery.

Rabbit A 243: weight 1785 gm., was used as the experimental animal. It received a total of 3.9 c.c. of thiocyanate solution. (1) Injected subcutaneously 0.092 gm. of morphine. (2) After fifteen minutes of narcosis 2.4 c.c. of 10 per cent solution of sodium thiocyanate was injected intravenously into the marginal ear vein. (3) After thirty-two minutes of narcosis the respiration was 30 per minute. (4) After one hour and three minutes 1.5 c.c. more of thiocyanate was injected. (5) After one hour and twenty minutes respiration was 46 per minute. (6) After three hours and twenty minutes the animal was up and hopped about but held its head with a 20 degree rotation to the right. (7) The next morning the animal was still quite weak and would not eat during the day. (8) The second morning the rabbit evidently had completely recovered. It was eating and quite active.

Although this rabbit did hop about a little sooner than the control, it showed signs of toxic effects for a day following the experiment and evidently barely escaped the fatal effects of the drug. The control had a respiratory rate through-

out the experiment which was from a third to a half again as rapid as that of the experimental rabbit. This showed depression of the respiratory center by the thiocyanate rather than a stimulation.

Of the three rabbits receiving the morphine and thiocyanate two died within forty-eight hours and the other barely escaped, although the dose was reduced, since it showed decided intoxication during the first twenty-four hours following the experiment.

CONCLUSIONS

1. The results obtained in experiments with sodium thiocyanate as an antagonist for ether did not corroborate the conclusions brought forth by Bancroft and Rutzler that thiocyanate ions antagonize the anesthetic action of ether. In a large majority of the ether experiments the return to normal of the lid reflexes and other manifestations required a longer time in these experimental animals than in their controls. In the quantities used seven out of seventeen rabbits which received the ether and thiocyanate died.

2. In experiments with sodium thiocyanate as an antagonist for sodium amytal it was found that the sodium thiocyanate did not shorten the long anesthesia but instead lengthened it, and decreased instead of increased the respiratory rate. Three of seven rabbits died.

3. In experiments with sodium thiocyanate as an antagonist for morphine, it was found that the morphine narcosis was not counteracted in any way, and that gradient reduction in the dosage of thiocyanate still proved fatal or toxic. Two of the three rabbits receiving the thiocyanate died.

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MICROSCOPIC SLIDE PRECIPITATION TESTS FOR THE DIAGNOSIS AND EXCLUSION OF SYPHILIS WITH SORE FLUID*†

Preliminary Report

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BASED upon 329 tests of varied sensitivity with various quantities of 126 fluids from 94 venereal and 12 nonvenereal sores with control clinical observations, blood tests, darkfield examinations and examinations of stained smears, the following microscopic slide precipitation tests for the diagnosis and exclusion of syphilis with sore fluid† are proposed:

Into each of two open slide chambers, 11 mm. in diameter, 0.025 c.c. sore fluid previously heated at 56° C. for one-half hour is pipetted. Additional specimens may be similarly pipetted into other chambers upon the same slide.

Into one-half of the chambers a very small drop (about 0.004 c.c.) of diagnostic test emulsion is allowed to fall from a capillary pipette.

Into each of the other sore fluids a very small drop of exclusion test emulsion is allowed to fall from a capillary pipette.

The slide is rotated on a flat surface with moderate vigor for five minutes.

The results are examined at once through the microscope at a magnification of about 120 times (16 mm. objective, 12× ocular) with the light cut down as for the study of urinary sediments and recorded in terms of pluses according to the degree of clumping and the size of the clumps.

The principles of the sore fluid tests described above are the same as those for the tests with heated serum.[1, 2]

Materials for the Microscopic Slide Precipitation Tests for Syphilis with Sore Fluid (see fig. 1)

1. *Sore Fluid.*—The lesion is thoroughly cleansed with sterile distilled water until all the pus, necrotic material and any local medication is completely removed. After proper cleansing, the clean base of the lesion is touched with a sterile platinum loop and the material obtained is used for a darkfield examination. The metal end of a 10 c.c. Record syringe barrel (see Fig. 1) is then adapted to the lesion and negative pressure produced with a small negative pressure pump (see Fig. 1). The suction is allowed to continue until after the darkfield examination has been made and recorded and then it is terminated. The three or four drops of sore fluid in the barrel are allowed to run into a capillary glass pipette 5½ inches in length, 3 mm. in diameter. The capillary pipette is

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Received for publication, February 10, 1932.

†Sore fluids turbid with exudate or containing any appreciable amount of local medication are unsatisfactory for testing. Admixture with blood, however, even in considerable quantity does not make the specimen unsatisfactory for the tests.

next touched to the base of the sore for additional fluid. If necessary, further suction is applied and the fluid obtained transferred to the capillary pipette. One end of the pipette is now sealed over a Bunsen burner. A second darkfield examination is then made and smears are prepared for special staining. The initial cleansing of the sore cannot be stressed too greatly since the presence of local medication, pus or debris in nonsyphilitic sore fluid may cause the precipitation of the antigen emulsion.

The sore fluid in the pipette is centrifuged for thirty to forty-five minutes at high speed (about 2000 r. p. m.) and the gross character of the fluid and the sediment noted. The pipette is then placed in a bath with water at a higher level than the top of the sore fluid and kept at 56° C. for one-half hour. After this the pipette is filed and broken just above the sediment and the fluid is allowed to run into or is drawn into a 0.2 c.c. pipette graduated in 0.001 c.c.

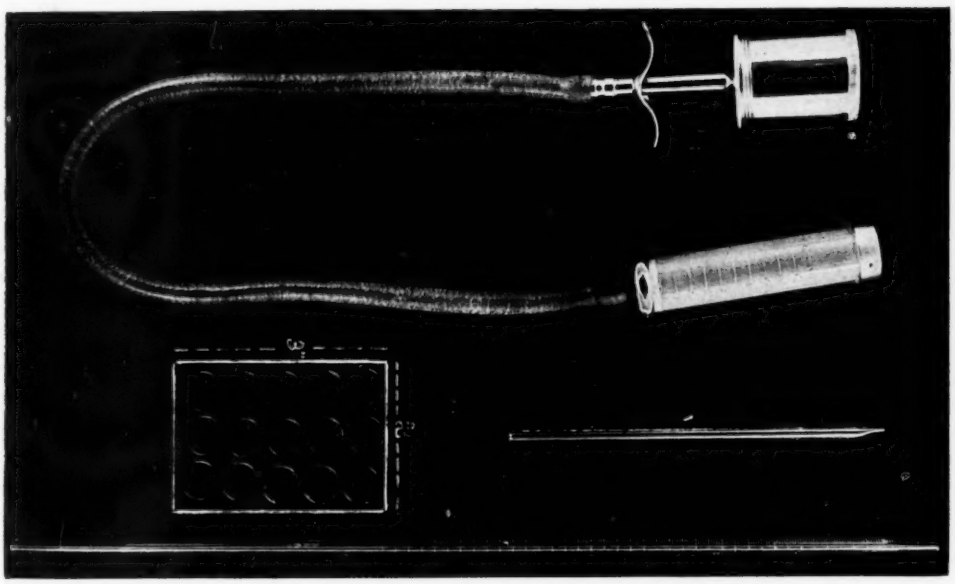

Fig. 1.—Materials for microscopic slide precipitation tests for syphilis with sore fluid.

The technic of obtaining and the preliminary handling of the sore fluid is much like that reported by Elliott and Todd.[3]

2. *Glassware.*—The pipette used to deliver the proper quantity of sore fluid is a 0.2 c.c. pipette, graduated in 0.001 c.c.

The pipettes for the antigen emulsion are Wright pipettes made from 6 to 10 mm. glass tubing drawn out into a very fine tube (about ¼ mm. in diameter).

Ordinary finely graduated 0.2 c.c., 1 c.c. and 10 c.c. pipettes are employed in the preparation of the antigen emulsions described below.

The antigen emulsions are prepared in ordinary cork-stoppered narrow mouthed 1 ounce bottles.

Microscopic slides 2 by 3 inches as purchased are rubbed on both sides with Bon Ami paste (prepared by allowing a cake of Bon Ami to remain in sufficient warm water to cover it for twelve hours or more. The paste keeps well, but may

require slight dilution with water from time to time). As soon as the paste is dry (in about five minutes), it is completely removed from the slide with a soft muslin cloth. For convenience the slides covered with paste may be stuck to each other, allowed to dry and cleaned at any time. Upon the clean slides, 15 paraffin rings, each with an inside diameter of 11 mm. are mounted. Upon slides so cleaned, sore fluid spreads freely. After use, the slides may be washed in hot water and prepared again as outlined above.

3. *Instrument for Making Paraffin Rings.*—This is essentially the instrument proposed by Green.[4] A piece of soft iron wire (No. 28) 14 cm. in length is wound twice tightly around a test tube about 12 mm. in outside diameter, forming a double loop and leaving a double shaft about an inch in length. The two shafts are then twisted together to within a quarter of an inch of the free end. After removing the looped wire from the test tube, a piece of linen thread (No. 12) about a yard long is started from the free end of the shaft after being fastened here by a single twist of the two free ends. Three long turns are made, reaching the loop which is then tightly wound with the thread; the winding is continued up the shaft to the free end where it is fastened between the two ends of the wire by twisting them. The loop is then bent at right angles to the shaft. It is then reshaped by working it against the bottom of the test tube mentioned above. The shaft is then inserted into the handle of a teasing needle or into a straight hemostatic forceps.

The paraffin rings are made by dipping the instrument into smoking paraffin (about 120° C.), draining quickly at one point, and transferring the remainder to the glass slide.

4. *Antigen.*—The purified antigen employed is one described previously.[5, 6] It is a lipid obtained from chilled absolute ethyl alcohol extract of beef heart muscle powder by precipitation in acetone at 50° to 37° C.

5. *Antigen Emulsions.*—The antigen emulsions for both diagnostic and exclusion tests have the following formula:

Formula:

0.85 c.c. distilled water (P_H about 6).
1.25 c.c. of 1 per cent cholesterin* (Pfanstiehl c.p.) in absolute ethyl alcohol (99+ per cent).
0.1 c.c. antigen.
2.2 c.c. of 0.85 per cent sodium chloride (c.p. or reagent, Merck) solution.

The technic of preparing the emulsion according to the above formula is as follows:

Into a 1 ounce bottle the required amount of distilled water (P_H about 6) is pipetted.

The bottle is held at an angle, and the 1 per cent cholesterin in absolute ethyl alcohol (99+ per cent) is allowed to run along the side of the neck of the bottle.

The bottle is gently rotated from the neck for twenty seconds.

*The 1 per cent cholesterin solution for the emulsions is prepared in about forty-five minutes by placing the cholesterin flakes and alcohol in a glass stoppered bottle in an oven at 50° to 56° C. and shaking gently a few minutes at fifteen minute intervals. The solution kept in the incubator at 37° C. is thoroughly satisfactory for use for as long as two months.

It is held at an angle again, and the proper amount of antigen is pipetted against the side of the neck of the bottle from a finely graduated pipette.

The bottle is promptly stoppered with a cork and shaken vigorously (the fluid thrown from bottom to cork and back) for one minute.

Lastly, the 0.85 per cent sodium chloride solution is allowed to run in quite rapidly, the bottle is stoppered again and shaken less vigorously than previously for one minute.

The emulsion, when examined under the microscope at a magnification of about 120 times, shows numerous very fine particles, but no clumps whatever.

For Diagnostic Test

One cubic centimeter or more of the emulsion in a narrow test tube (about 12 mm. inside diameter) is placed in a water-bath at 35° C. for fifteen minutes. The emulsion as soon as heated is ready for use.

For Exclusion Test

Two cubic centimeters of the emulsion in a narrow test tube (about 12 mm. inside diameter) is placed in a water-bath at 56° C. for fifteen minutes, after which it is poured into a 3 by 1 inch tube and centrifuged for fifteen minutes (eighth setting of Rheostat of International Equipment Company Centrifuge, size 1, type SB about 2000 r. p. m. unloaded).

After centrifugation, the fluid is decanted and with the tube inverted, the inside is dried with a cloth almost to the level of the sediment.

To the sediment 3 c.c. of 0.85 per cent sodium chloride solution is added.

The sediment is distributed throughout the fluid by shaking and then poured into a narrow tube for use.

These emulsions, kept at room temperature, are satisfactory for at least twenty-four hours after preparation.

COMMENT

The majority of sore fluids were obtained from patients in the venereal wards of City Hospital and Lakeside Hospital through the kindness of H. N. Cole. The remainder were obtained from patients attending Mount Sinai Hospital and Charity Hospital. Those from the latter hospital were obtained through the courtesy of C. G. LaRocco. A few were obtained from patients of S. L. Bernstein and J. R. Breitbart and thanks are due for their kind cooperation.

A summary in brief of the sore fluids examined follows:

Total Specimens Examined		126
Total Tests (with various quantities of sore fluid and with emulsions of varying sensitivity)		329
Total Cases		106
A. Total Venereal Cases:		94 as follows:
1. Active syphilitic		36
(a) Primary	18 Genital chancres (1 associated with chancroid)	
(b) Secondary	17 Genital or anal condylomas or papillomas	
(c) Congenital	1 Bulla	
2. Chancroid		54
3. Granuloma inguinale		3
4. Lymphogranuloma inguinale		1

B. Total Nonvenereal Cases:		12 as follows:
1. Impetigo	2	
2. Vincent infection	1	
3. Molluscum contagiosum	1	
4. Furuncle	1	
5. Infected scabetic lesion	1	
6. Herpes	1	
7. Actinomycosis	1	
8. Erythema multiforme	1	
9. Rodent ulcer	1	
10. Indurated genital lesion, 1 year's duration (undiagnosed)	1	
11. Indurated genital lesion, 14 years' duration (undiagnosed)	1	

Table I shows the results of active syphilitic sore fluid and blood examinations in the active syphilitic cases.

In each case, a record was kept of the clinical data including the character of the sore and other related structures, the time of appearance, the times of exposure, the antiseptic, if any applied, the reaction of the patient at the time the sore fluid was obtained, etc. In each instance the gross and microscopic character of the sore fluid was noted. A darkfield examination or an examination of a smear of the sore fluid stained by the Fontana method was made in each instance, usually both. When indicated smears were prepared by special stains and examined for Ducrey's bacillus and Donovan bodies. To control the sore fluid results, blood withdrawn from an arm vein at the time the sore fluid was obtained was tested for syphilis by the microscopic slide precipitation and Wassermann methods. In the majority of cases a spinal fluid examination including a Wassermann test was likewise made. All of the patients were followed for a sufficient period of time to rule out any masked infection.

In the beginning of the study, fluid was obtained from the sore by using firm pressure of gloved fingers on both sides of it and was allowed to run into a capillary pipette. This method caused much pain and discomfort to the patient and required a long time to obtain more than a drop or two. In the course of time, the technic described above was developed by which the patient was caused little or no discomfort and the fluids obtained for testing were thoroughly satisfactory in quantity and character.

At first, 0.01 c.c. of sore fluid was employed in the open slide precipitation tests. With the development of a satisfactory method of obtaining larger quantities of fluid 0.025 and 0.05 c.c. were used. When 0.01 c.c. was tested, a similar quantity of antigen emulsion was added. The microscopic slide precipitation tests with blood obtained at the same time gave so much stronger results with the same emulsions in positive cases that it was considered advisable to change the conditions of the sore fluid test. By employing 0.025 and 0.05 c.c. of sore fluid and by the use of very small or small drops of antigen emulsion equal to about one-sixth the quantity of sore fluid, results more comparable with those of the blood were obtained. In the great majority of instances however with conditions quite comparable and with the same (diagnostic blood test) antigen emulsion, the blood gave decidedly stronger reactions than did the sore fluid. For this reason, the use of the more sensitive emulsion described above for the diagnosis of syphilis with sore fluid is justified. Likewise, with condi-

TABLE I

DATE	PATIENT	LUETIC LESION	DURATION	SORE FLUID				SORE FLUID		SORE FLUID QUANTITY OF (HEATED AT 56° C. ½ HOUR) IN TEST	EMULSION EMPLOYED IN SORE FLUID TEST										
				TURBID WITH		TURBID WITH		DARK FIELD	DARK FIELD	FONT-ANA	FONT-ANA		C 7½ 35	C 10 35	C 10 56	C 12½ 35	C 12½ 45	C 12½ 56	C 10 35c	C 10 56c	C 12½ 35c	C 12½ 45c	C
				LITTLE BLOOD	MUCH BLOOD	LITTLE EXUDATE	MUCH EXUDATE																
10/22/30	W. W.	Chancre	2 weeks				+		−		−	0.01		−									
10/29/30	L308-413	Chancre	10 days		+			+		+		0.01		−									
11/ 5/30	J. L.	Chancre	5 weeks	+				+			−?	0.01					+++					++++	
12/ 3/30	A. K.	Chancre	6 days	Clear				+		+		0.01						++++			++++		
12/10/30	R. S.	Chancre	6 weeks	+					−		−	0.01				++++		++++			++++		
12/10/30	J. M.	Chancre	3 weeks	Clear				+			−	0.01				−		±					
12/17/30	J. P.	Chancre	1 week			+		+		+		0.01		−		−							
														−		++							
12/17/30	L312-226	Chancre	2 weeks				+	+		+		0.01							++++		++++		
1/20/31	I. L.	Chancre	2 weeks				+		−		−	0.025				+++		++++					
																++++							
1/27/31	F. S.	Chancre	3 weeks	+					−		−	0.025		++++		++++							
1/30/31	J. B.	Chancre	6 days	+				+		+		0.025		−		−			−				
1/30/31	J. L.	Chancre	5 days	+				+			−	0.025		−		−							
1/30/31	F. J.	Chancre and chancroid	3 weeks	+					−		−	0.025		++++		++++							
2/11/31	E. R.	Chancre	3 weeks	Moderate turb.				+		+		0.025		++									
4/17/31	L. F.	Chancre	9 days	+						+		0.025		−									
												0.05		−									
6/ 2/31	J. L.	Chancre	2 months	+				+				0.025		++++						++++			
10/30/31	M. G.	Chancre	10 days		+					+		0.025		−									
11/14/31	C. A.	Chancre	3 weeks	Moderate turb.				+		+		0.025				++++				++++			
												0.05		++++									
10/22/30	P. W.	Condyloma		+				+		+		0.01		++++									
10/29/30	B. B.	Condyloma			+			+		+		0.01		−						+++			
11/ 5/30	M. G.	Condyloma		+		+		+		+		0.01											
11/12/30	R. J.	Condyloma		+				+		+		0.01						−					
11/26/30	F. H.	Condyloma		+				+		+		0.01									−		
12/ 3/30	F. K.	Condyloma		Clear				+		+		0.01						−			++		
12/ 3/30	E. L.	Condyloma				+		+		+		0.01						−			−		
12/17/30	E. C.	Condyloma			+			+		+		0.01							++++		++++		
1/27/31	L. A.	Condyloma		+				+		+		0.025	±	++++		++++							
1/27/31	E. S.	Condyloma		+				+		+		0.025	−	±		++							
1/27/31	J. M.	Condyloma		+				+		+		0.025	±	++++		++++							
1/27/31	R. S.	Condyloma			+			+			−	0.025		±, +		++, +++							
1/30/31	M. M.	Condyloma		+				+		+		0.025		++++		++++							
3/17/31	J. K.	Condyloma		+				+		+		0.025		++++	++++								
3/17/31	J. C.	Condyloma		+				+			−	0.025		−	++++			++++					
4/ 3/31	A. H.	Condyloma					+	+		+		0.025		++++				++++					
												0.05			++++								
4/14/31	Mc. I.	Condyloma		+				+		+		0.025		++++				++++					
												0.05			++++								
4/17/31	J. E.	Bulla		+				+			−	0.025			++++			++++					
Total		36																					

Emulsions:
C = Cholesterin
c = Centrifugated

Number below C = degree Centigrade at which emulsion heated
Number after C = c.c. (×10 of cholesterin)
Diag. = Diagnostic

Excl. = Exclusion
S = Sensitive
VS = Very Sensitive

TABLE I

SORE FLUID				SORE FLUID QUANTITY OF (HEATED AT 56° C. ½ HOUR) IN TEST	EMULSION EMPLOYED IN S						
DARK FIELD	DARK FIELD	FONT-ANA	FONT-ANA		C 7½ 35	C 10 35	C 10 56	C 12½ 35	C 12½ 45	C 12½ 56	C 3
	−		−	0.01		−					
+		+		0.01		−					
+			−?	0.01					+++		
+		+		0.01						++++	
	−		−	0.01				++++		++++	
+			−	0.01				−		±	
+		+		0.01		−		−			
						−		++			
+		+		0.01							++
	−		−	0.025				+++		++++	
								++++			
	−		−	0.025		++++		++++			
+		+		0.025		−		−			
+			−	0.025		−		−			
	−		−	0.025		++++		++++			
+		+		0.025		++					
		+		0.025		−					
				0.05		−					
+				0.025		++++					
		+		0.025		−					
+		+		0.025				++++			
				0.05		++++					
+		+		0.01		++++					
+		+		0.01		−					
+		+		0.01							
+		+		0.01						−	
+		+		0.01						−	
+		+		0.01						−	
+		+		0.01							++
+		+		0.025	±	++++		++++			
+		+		0.025	−	±		++			
+		+		0.025	±	++++		++++			
+			−	0.025		±, +		++, +++			
+		+		0.025		++++		++++			
+		+		0.025		++++	++++				
+			−	0.025		−	++++			++++	
+		+		0.025		++++				++++	
				0.05			++++				
+		+		0.025		++++				++++	
				0.05			++++				
+			−	0.025			++++			++++	

at which emulsion heated
esterin)

Excl. = Exclusion
S = Sensitive
VS = Very Sensitive

LOYED IN SORE FLUID TEST							HEATED SERUM TESTS						
							SLIDE TEST		WASSERMANN				
2½ 5	c 12½ 56	c 10 35c	c 10 56c	c 12½ 35c	c 12½ 45c	c 12½ 56c	DIAG. c 10 35c	EXCL. c 10 56c	S	VS	OUTSIDE WASS.	SPINAL FLUID WASS. OUTSIDE	MEDICATION LOCAL
							++++	++++	+	−	+++	−	None
							−	−	−	−	−		None
++					++++						++++	−	None
	++++			++++			++	+++	++++	++++	−		None
	++++			++++			++++	++++	++++	++++	++++	−	Pot. Permang.
	±						++++	++++	++++	++++	++++	−	None
							++++	++++	++++	++++	+++		None
		++++		++++			++++	++++	++++	++++	++++		Ointment (kind?)
	++++						−	±	−	−	+, ++	−	Saline
							++++	++++	++++	++++	++++	+++	None
		−					−	±, +	−	−	−		None
							−	+++	−	−	−		None
							++++	++++	++++	++++	++++		Saline
							++++	++++	++++	++++	++++	−	Ammon. merc.
						−	−	++	−	−			None
			++++				++++	++++	+++	++++	++++	−	None
							−	±					Calomel powder
			++++				++++	++++			++++	−	Saline
							++++	++++	+++	−?	++++		
			+++				++++	++++	AC	AC	++++		
							++++	++++	++	±	++++	−	
	−					−	++++	++++	++++	++++	++++		
				−		−	++++	++++	++++	++++	++++		
	−			++			++++	++++	++++	++++	++++	−	
	−			−			++++	++++	++++	++++	++++	−	
		++++		++++			++++	++++	++++	++++	++++		
							++++	++++	++++	++++	++++		
							++++	++++	++++	++++	++++		
							++++	++++	++++	++++	++++		
							++++	++++	++++	++++	++++	−	
							++++	++++	++++	++++	++++		
							++++	++++	++++	+++	++++	−	
	++++						+++	++++	++++	++++	++++	−	
	++++						++++	++++	++++	++++	++++	−	
	++++						++++	++++	++++	++++	++++	−	
	++++										++++		

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tions quite comparable, the exclusion slide test emulsion for blood gave much more strongly positive results with blood than with sore fluids, justifying the use on tests with sore fluids of the more sensitive exclusion test emulsion described above. In only one instance did chancre fluid give a positive reaction when the blood was negative. The fluid was turbid with exudate and the significance of the result therefore is questionable. In the course of the study, eleven antigen emulsions of varied sensitivity were employed, the best results being obtained with the emulsions recommended.

A number of tests were done with unheated sore fluids, with and without the addition of hypertonic salt solution as employed in unheated serum tests.[7] Although the results were frequently satisfactory even without the addition of salt solution, the impossibility of determining the optimal quantity of electrolytes to add to each specimen and the fact that small quantities of electrolytes increase the agglutinating capacity of unheated serum much more than that of heated serum has led to the impression that more reliable results may be expected if the sore fluids are heated at 56° C. for one-half hour before they are tested.

It was observed that nonspecific sore fluids turbid with exudate frequently gave positive results. Likewise nonsyphilitic sore fluids containing any appreciable quantity of antiseptic soaps, solutions, salves, etc., usually precipitated the antigen emulsions. Accordingly such fluids are unsatisfactory for testing for the reagin of syphilis. It was found, however, that when blood even in considerable quantities was present, the results in syphilitic and nonsyphilitic cases were satisfactory. In fact it is quite possible that the positive results of sore fluid tests depend upon the presence of blood squeezed from injured vessels rather than upon reagin in an extravascular fluid in the sore itself. This impression is strengthened by the fact that blood tinged and bloody chancroidal sore fluids obtained from cases having tertiary syphilis gave positive precipitation results (blood drawn from an arm vein at the same time likewise gave positive results) in contrast to negative reacting chancroidal fluids obtained from individuals free of syphilis.

More experience will be required to properly evaluate the sore fluid tests. It is quite apparent however that in general sore fluid does not compare favorably with blood as a specimen for testing for syphilis, since even small quantities of such adventitious substances as antiseptics, inflammatory exudates, products of degenerating tissue, etc., may cause it to give false positive results. Furthermore there is some evidence that the sore fluid results in specific cases depend upon the presence of blood squeezed from injured vessels in the walls of the sore and that better results are obtainable by tests of blood drawn directly from a large vein.

SUMMARY

Based upon 329 tests of varied sensitivity with various quantities of 126 fluids from 94 venereal and 12 nonvenereal sores with control clinical observations, blood tests, darkfield examinations and examinations of stained smears, the microscopic slide precipitation tests for the diagnosis and exclusion of syphilis with sore fluids described above are proposed.

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LABORATORY METHODS

ELEMENTAL IODINE*

A New Technic for the Evaluation of Its Fungicidal Properties

Albert Strickler, M.D., Philadelphia, Pa.

INTRODUCTION

In MODERN medicine new therapeutic suggestions do not rest on empiricism alone, but are founded on facts revealed through investigative laboratory methods. The efficiency of iodine as a fungicide has been known and its place among this class of remedies is quite secure. Empiricism first and subsequently favorable clinical results have been responsible for faith in iodine. Although it had long been known that iodine vaporized very readily, application of iodine vapor to the skin has not been medically used, but instead, various preparations of iodine as the tincture of iodine, Lugol's solution and the official Unguentum Iodi have been introduced and popularized. Theoretically, these combinations do not add strength to the iodine, but on the contrary, probably lessen its efficiency. In the tincture of iodine, the addition of the alcohol hardens tissues by absorbing the water and precipitating the proteins,[1] a condition which should interfere with the penetration and activity of the iodine. The strength of the tincture of iodine (U. S. P.) is only 7 per cent. The iodine ointments are weak insofar as iodine activity is concerned. They do not react readily with proteins, as they give up their iodine slowly, owing to previous chemical combination of the iodine with the bases of the ointment preparations. The official iodine ointment consists of 4 per cent each of iodine and potassium iodide in glycerin and wool fat.

It is evident that both theoretically and practically, the commonly used iodine compounds are not ideal fungicides. These preparations of iodine do not utilize 100 per cent of this agent, the tincture being a 7 per cent preparation, and the official ointment a 4 per cent combination. In the tincture, as previously stated the alcohol tends to limit the penetrability of the iodine and increases its tendency for skin irritation, while in the official ointment, its combination with fatty bases tends to decrease markedly iodine activity. The ideal fungicide should possess the following qualities: penetration; prolonged fungicidal activity; minimum reaction with the cutaneous tissues; and specific chemiotaxis for the fungus protoplasm.

The introduction of chemotherapeutic methods has opened a new avenue for the exact evaluation of the old fungicidal agents and has offered hope for the discovery of the ideal remedy.

*From the Pathological and Chemical Laboratories, The Skin and Cancer Hospital of Philadelphia, Albert Strickler, M.D., Medical Director.
Received for publication, January 15, 1932.

PREVIOUS FUNGICIDAL EXPERIMENT

The earliest of these attempts was the investigation of Schamberg and Kolmer,[2] in which the fungistatic and fungicidal activity of various dyes and medicaments were studied. Their method consisted of placing in each test tube 4 c.c. of Sabouraud's maltose agar titrated to 1 per cent acid to phenolphthalein. The dyes and medicaments were prepared in 1:100 solution or suspension in sterile distilled water, and higher dilutions were prepared in sterile test tubes in amounts of 1 c.c. The agar, and solution or suspension of dye or medicament were mixed, slanted, and allowed to harden. These tubes were then seeded with the respective cultures of the Trichophyton rosaceum and Microsporon audouini, and Achorion Schoenleinii. The tubes were kept at room temperature. The results were read at weekly intervals for six to eight weeks.

In this investigation, twenty-one compounds were studied including iodine, mercuric chloride, calomel, salicylic acid, benzoic acid, sodium hyposulphite, etc. As a result of this study, the authors concluded that "Iodine transcends all other medicaments in restraining the growth of certain moulds, but in aqueous solution it is inferior to certain other drugs in killing them." The strength of the iodine used in these experiments was 1:2000 of a 2 per cent solution (2 gm. iodine crystals; 5 gm. potassium iodide and 100 c.c. water) = 1:100,000 of pure iodine.

The pioneer researches of Schamberg and Kolmer seemed to focus attention upon iodine and its compounds as offering the most fertile basis for the uncovering of the ideal fungicidal agent. Following these investigations, came the work of Sharlit and Highman[3] who proposed the use of a compound of iodine first described by Horton in 1888, and known as tetraiodomethenamine $(CH_2)_6N_4I_4$. Being an unstable iodine compound, it was found capable of giving off free iodine vapor very much after the manner of iodine crystals, only more slowly. The reasons advanced by the author for proposing this iodine compound were that, "Iodine as a fungicide should be most efficacious in the treatment of patients who have mycotic dermatoses, not alone for its high fungicidal coefficient, but also because iodine, extremely volatile at ordinary temperature, may be fed to the skin as a gas and as such has a fair measure of penetrability. A constant stream of iodine acting over a long period of time, reaching the skin in fungicidal strength yet not irritating the skin is a desideratum." Here we have for the first time an inkling that iodine vapor applied to the skin may possess superior fungicidal properties.

In order to determine experimentally the relative fungicidal efficiency of tetraiodomethenamine and of iodine, the authors set up a number of tests as follows: Varying concentrations of tetraiodomethenamine and of iodine were added by constant volume to Sabouraud's media and these were slanted and hardened before being seeded with cultures of the Microsporon lanosum. They were incubated at room temperature. To render both the free iodine and the tetraiodomethenamine water soluble, potassium iodide was added to each of the solutions. The growths were examined at the end of two weeks. These experiments seemed to show the superiority of the tetraiodomethenamine over iodine, inasmuch as the iodine in both a 40:100,000 and a 20:100,000 dilution

failed to inhibit the fungus growth, while in those same dilutions, tetraiodomethenamine did have fungistatic action.

Another experiment devised by the authors for study of the fungistatic action of these two preparations consisted in taking bits of masses of fungi about pea size, soaking them in 1 c.c. of an aqueous solution of iodine and of tetraiodomethenamine respectively for forty-eight hours, seeding on appropriate media and growing at room temperature. These experiments showed

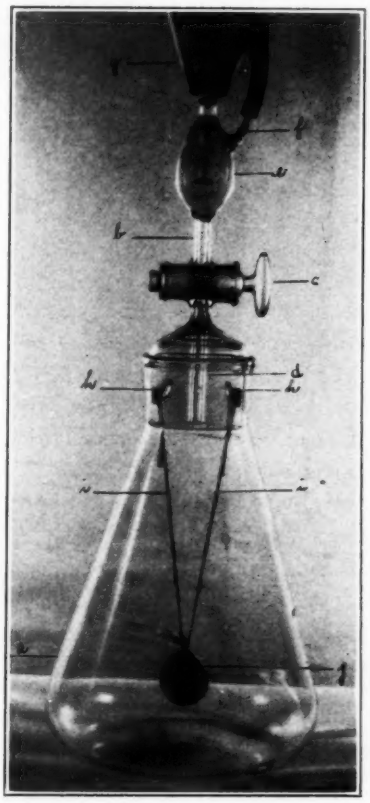

Fig. 1.

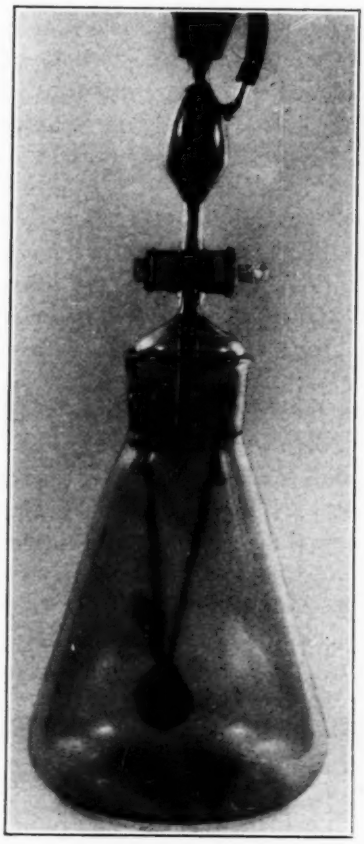

Fig. 2.

Fig. 1.—The Strickler-Borneman apparatus. *a*, Pyrex flask, *b*, neck ground to fit air-tight with inverted glass cup part, *c*, stop-cock, *d*, central glass tube, *e*, bulb-like expansion, *f*, hollow glass side arm, *g*, vacuum vaporizer, *h*, two glass hooks, *i*, threads, *j*, collodion sac.
Fig. 2.—"The Strickler-Borneman Apparatus" after iodine vapor treatment.

equal fungistatic powers for both the aqueous iodine plus potassium iodide combination and for the tetraiodomethenamine plus potassium iodide mixture.

The conservative interpretation placed upon the above experiments is an acknowledgement of the marked difference between the iodine laboratory experiments and application of this remedy to the human skin in disease.

"The Strickler-Borneman Apparatus." Description: Clinical use of "The Henal Vacuum Vaporizer"[4] with iodine as the medicinal agent prompted the author to devise a laboratory method for testing the fungicidal powers of iodine vapor in a manner comparable to its actual use on the human diseased skin.

To meet this condition "The Strickler-Borneman Apparatus" was devised. It consists of a pyrex flask (*a*) of about 1 liter capacity with the neck so ground as to fit air-tight with the inverted glass cup part (*b*) provided with a stop-cock (*c*). This inverted cup-shaped portion has a central glass tube (*d*) throughout its length for the passage of the iodine vapor; it also houses the stop-cock. The upper terminus of the cup-shaped portion consists of a bulb like expansion (*e*) with a hollow glass side arm (*f*) so arranged as to fit "The Henal Vacuum Vaporizer" (*g*). On the inner surface of the cup-shaped portion (*b*) and as close to the neck of the flask (*a*) as possible there are two glass hooks (*h*) one in each side to hold the threads (*i*) which suspend the collodion sac (*j*) loaded with the culture to be tested. Before each experiment a small amount of sterile petrolatum is applied to the ground fitting surfaces of "a" and "b" to insure air-tight contact.

TECHNIC

Collodion sacs were made by pouring several cubic centimeters of Merck's flexible collodion into large test tubes and distributing evenly over the surface. The test tubes were inverted to permit escape of the excess collodion making possible preparations of sacs of fairly uniform thickness. After allowing the collodion solvent (alcohol-ether) to evaporate spontaneously, the sacs were immersed in water to facilitate their removal from the tubes. Each sac was tested for leaks just prior to use to avoid the possibility of iodine vapor passing through small holes. The average thickness of the sacs was 0.01 to 0.02 mm. Tests with "The Henal Vacuum Vaporizer" showed that 0.0175 gm. of iodine were delivered on each application and that two such applications were required to fill the flask with vapor.

The medium for growing the ringworm fungi consisted of peptone (French) 10 gm.; crude dextrose 40 gm. water 1 liter (Weidman's Pennsylvania Medium without agar). The fungi were grown in this medium for approximately two weeks. Five cubic centimeters of the suspension of the organism tested was placed in a collodion sac the open end of which was tied with a sterile string and the end sealed with fresh collodion so that no vapor could penetrate except through the walls of the sac. The constant volume of suspension used made the exposed surface fairly constant so that differences occurring could not be due to variation in the surface exposed to the iodine vapor. Tests with the sac filled with suspension of the organism (ringworm fungus) and allowed to stand for one hour showed that the sac itself had neither lethal nor inhibitory effects upon the fungus in the absence of iodine vapor.

In conducting these experiments, the sealed collodion sacs containing suspension of the fungus tested were suspended in "The Strickler-Borneman Apparatus" and subjected to two applications of iodine vapor by means of "The Henal Vacuum Vaporizer" using a negative pressure of 15 cm. After permitting the sacs to remain in the iodine atmosphere for varying periods of time they were removed and opened. Samples were obtained with sterile pipettes and transferred to culture tubes containing Weidman's Pennsylvania Medium. These were observed for varying periods of time as detailed in the schedules below. The control cultures were removed before subjection to iodine vapor.

TABLE I

FUNGISTATIC AND FUNGICIDAL RESULTS WITH IODINE VAPOR USING "THE HENAL VACUUM VAPORIZER" AND "THE STRICKLER-BORNEMAN APPARATUS"

NAME OF FUNGUS	DATE OF EXPERIMENT	CONTROL	DURATION OF IODINE VAPOR EXPOSURE			
			5 MIN.	15 MIN.	30 MIN.	60 MIN.
Aspergillus Niger	8/10	8/11 growth			8/11 growth	8/11 less growth than 30 min.
Trichophyton gypseum pleomorphic	8/11	8/17 growth	8/17 growth	8/17 no growth	8/17 no growth	
Cryptococcus castellanei	8/12	8/17 growth	8/17 growth	8/17 growth	8/17 growth	
Microsporon audouini	8/14	8/17 growth		8/17 no growth	8/17 no growth	8/17 no growth
Epidermophyton inguinale	8/14	8/17 growth		8/17 growth	8/17 no growth	8/17 no growth
Trichophyton asteroides	8/20	8/26 growth		8/26 no growth	8/26 no growth	8/26 no growth

These cultures were observed for 5 days then formalized.

Recognition of the possibility of delayed growth prompted repetition of these experiments. In Table II the cultures were observed for twenty-two days.

TABLE II

FUNGICIDAL RESULTS WITH IODINE VAPOR USING "THE HENAL VACUUM VAPORIZER" AND "THE STRICKLER-BORNEMAN APPARATUS"

NAME OF FUNGUS	CONTROL	TIME OF EXPOSURE AND RESULTS	
		30 MINUTES	60 MINUTES
Microsporon audouini			
began growth	3rd day	5th day	no growth
reached maximum	7th day	21st day	no growth
Epidermophyton inguinale			
began growth	5th day	no growth	no growth
reached maximum	13th day	no growth	no growth
Trichophyton asteroides			
began growth	3rd day	no growth	no growth
reached maximum	12th day	no growth	no growth

Figs. 3 and 4 depict the results of exposure of ringworm cultures to iodine vapor in "The Strickler-Borneman Apparatus" after reculture.

Desire to determine whether iodine crystals as such, or, as iodine vaporized, were responsible for the results obtained in the above experiments prompted the setting up of another series of fungicidal tests. Three different strains of ringworm fungi were grown in Weidman's Pennsylvania Media, without agar for two weeks; they were the Microsporon audouini; the Trichophyton asteroides; and Epidermophyton interdigitale. To each of the collodion sacs containing 5 c.c. of the above ringworm culture suspensions, iodine crystals in the following respective amounts were added: i. e., 0.00103 gm.; 0.00216 gm.; and 0.00388 gm. These amounts of iodine were determined as present within the collodion sacs

after their exposure to iodine vapor for 15 min., 30 min., and 60 min. respectively, using the Strickler-Borneman apparatus and the Henal Vacuum Vaporizer. The contents of the sacs were thoroughly agitated and mixed and kept at room temperature for fifteen minutes, thirty minutes, and sixty minutes, respectively. The amounts of iodine and the time exposures were identical with those used in the conduct of "The Henal Vacuum Vaporizer" and "The Strickler-Borneman Apparatus" fungicidal tests. At the expiration of the respective time limits, each of the cultures was transplanted by placing a few drops of the iodine treated suspension on Pennsylvania agar slants. Daily observations were made and the first indications of growth appeared from the third to the seventh

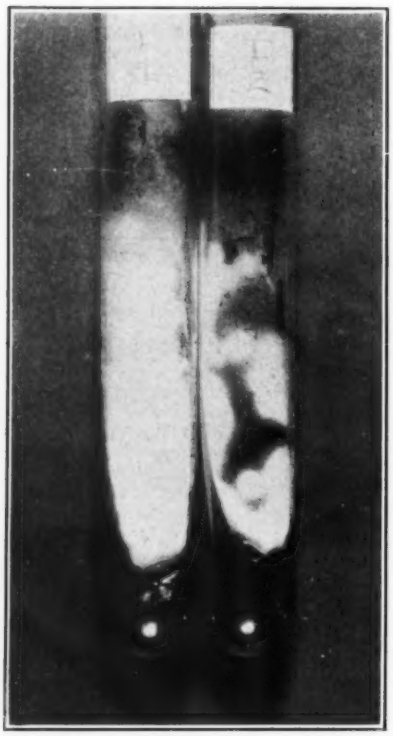

Fig. 3.

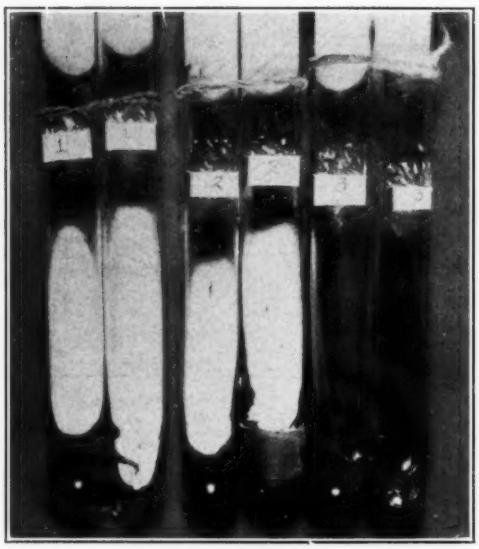

Fig. 4.

Fig. 3.—Microsporon audouini. *1*, Control, *2*, delayed growth. Iodine vapor fifteen minutes.

Fig. 4.—Microsporon audouini. *1*, Controls, *2*, iodine vapor thirty minutes, *3*, iodine vapor sixty minutes.

day. This observation period lasted three weeks. The results showed that the growth in the iodine-treated cultures almost equaled that of the control, so that iodine crystals per se, as used in the above manner seem to have failed to retard growth of the ringworm fungi.

COMMENT

The experiments here cited warrant the following comments:

1. "The Strickler-Borneman Apparatus" and the technic suggested, offer a new method of testing the fungicidal and fungistatic action of iodine vapor, through interposition of a nonabsorbable membrane between the fungi and

the medicament and through use of the agent tested in the form identical with that applied to the human skin. No doubt the same technic could be applied to other vapors and gases. The identical technic has been used for testing the bacteriocidal properties of iodine vapor, and upon this phase, a report will be made shortly.

2. The laboratory experiments proposed simulated closely conditions of treatment adopted for epidermophytosis and for tinea tonsurans in the human being.

3. Proof has been furnished of the remarkable fungicidal properties of iodine vapor, confirming the suggestive conclusions of other authors.

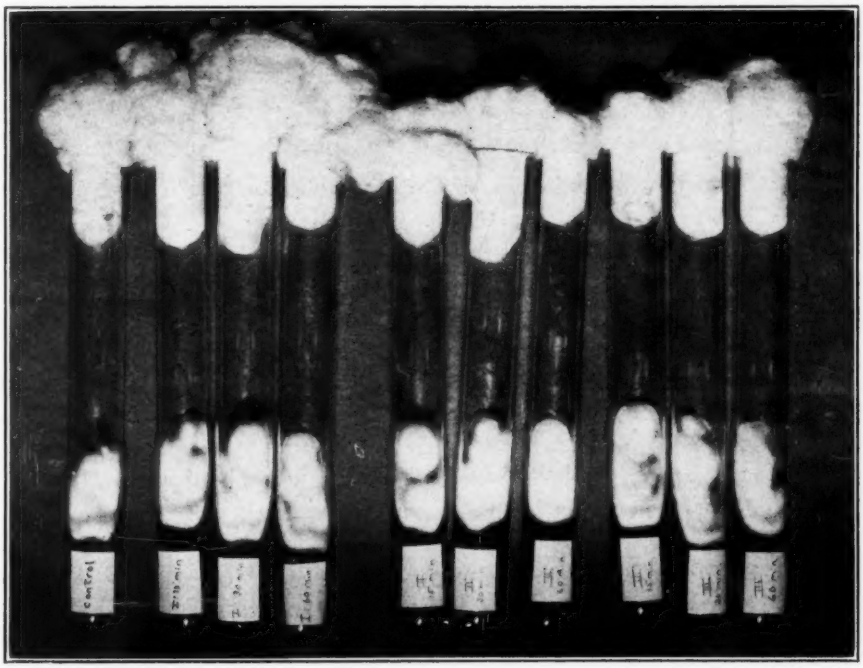

Fig. 5.—Epidermophyton interdigitale agar cultures after treatment with iodine crystals. Note: Growth in treated tubes, practically equals growth in control tube.

4. The cultures observed for only five days after treatment with the iodine vapor gave, in the main, identical results as those observed for twenty-two days, showing absence of delayed growth and proving that iodine vapor really possesses fungicidal action.

5. Experiments are now being conducted with the thought of formulating means for prolonging iodine activity and for increasing its chemotropism for the ringworm fungi. As this work is far advanced, a report of these findings will be released in the very near future.

ADDENDUM

Since writing this paper, a contribution by Schamberg and his associates has appeared in the December, 1931, issue of *The Archives of Dermatology* in which they state that metallic iodine appears to be the strongest present day

fungicide. They based this statement on certain experiments conducted with Lugol's solution.

The author wishes to express his appreciation to Mr. George Borneman of Millville, New Jersey for his efforts in the construction of "The Strickler-Borneman Apparatus."

The technical part of this investigation was carried out by Frederick Summerill, M.D., and Philip D. Adams, M.S., of the Research staff of The Skin and Cancer Hospital of Philadelphia to whom the author wishes to express his appreciation.

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806 PINE STREET.

THE WIDAL TEST IN TUBERCULOSIS*

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DURING the past year two instances have come to my attention in which a positive macroscopic agglutination test was obtained for typhoid fever in a titer (1-320) usually regarded as diagnostic for that disease, although the patients had no clinical symptoms of typhoid fever. They did however, have tuberculosis. In one case the infection was pulmonary, in the other, renal. One patient gave no history of either typhoid fever or antityphoid vaccination. The other patient had been vaccinated twelve years previously but there was no history of typhoid fever. On inquiry among clinicians primarily interested in tuberculosis, another instance of this kind was found and the general impression appeared to be that positive Widal tests were occasionally encountered in tuberculous infection, and but little attention was paid to them.

In 1923, Hull and Henkes[1] reported positive microscopic Widal tests (serum dilution 1-40) in 53 of 100 specimens of blood from early tuberculosis and in 1 of 13 advanced cases. The macroscopic tests on 12 specimens were negative although 8 of these were positive by the microscopic method. Hull and Henkes[2] also have studied agglutination of B. dysenteriae Flexner by blood serum from persons in the incipient stage of tuberculosis, and obtained 75 per cent positive results. They state that no agglutination was obtained with blood from cases of advanced tuberculosis or from normal individuals. Kilduffe and Hersohn[3] have made similar observations. Since the completions of my studies, Madgwick and Partner[4] have published a paper dealing with serum reactions in tuberculosis.

It seemed desirable to make a further study of this reaction, particularly in the light of the recent work of Felix,[5] Craigie,[6] and Eldering and Larkum,[7] with the so-called "H" and "O" types of agglutination. Since my two instances of "false" positives were in persons having advanced tuberculosis, this type of in-

*Department of Health.
Received for publication, February 22, 1932.

fection was studied. Blood was obtained from 61 hospital patients,* all adults, 50 men and 11 women. The Kahn test was positive in 4 persons; negative in 53; not made in 4. In 7 instances there was a definite history of typhoid fever, the most recent being 11 years ago. Nine had received antityphoid vaccination.

The agglutination tests were made macroscopically, using serum dilutions ranging from 1-20 to 1-320. Two formalized typhoid antigens were used. One was a Rawlings strain. This strain is commonly used for Widal tests in diagnostic laboratories. Our culture gave an "H" or flocculent type of agglutination. The other antigen was obtained through the courtesy of Dr. N. W. Larkum of the Michigan State Department of Health and was marked "O" 901.[8] This culture was rich in "O" antigen and gave the characteristic "O" type agglutination. In addition, B. paratyphosum A and B antigens were used.

No agglutination was obtained in any serum with either of the paratyphoid cultures. The "H" antigen (Rawlings strain) and the "O" antigen gave results as shown in Table I. The other 53 serums did not agglutinate either the "H" or "O" antigens. Kahn tests on the eight serums which agglutinated the "H" type culture were all negative.

TABLE I

TITER AGAINST ANTIGEN		HISTORY OF	
"H"	"O"	TYPHOID FEVER	ANTITYPHOID VACCINATION
1 - 20	none	Yes	Yes
1 - 40	none	Yes	No
1 - 40	none	No	No
1 - 40	none	No	?
1 - 80	none	No	No
1 - 80	none	No	No
1 - 160	none	No	Yes
1 - 160	none	No	Yes

It is apparent that the Rawlings strain of the typhoid baccillus may give the flocculent type of agglutination in the blood serum of patients ill with tuberculosis, but having no clinical signs of typhoid fever. In some instances (4 of 61 in this series), the titer fell in a range usually considered of diagnostic significance. There was no relation in this small series between such titer and the history of an attack of typhoid fever or of antityphoid vaccination. It is admitted that previous typhoid infection in any group cannot be absolutely ruled out. Since all of the serums which agglutinated the "H" antigen failed to agglutinate the "O" antigen, this latter type of agglutination must be considered in interpreting the results of Widal tests in advanced tuberculosis. These data emphasize the relative value of the two antigens in ruling out typhoid infection as a complication in that disease. It may not be out of place to point out that anomalous serologic results have not infrequently been reported in connection with tuberculosis. False positive Wassermann tests have been encountered, although Kolmer[9] believes the results are due to technic; agglutination reactions in blood serum with typhoid and dysentery bacilli have been observed; and Pinner and

*The specimens of blood and histories of patients were obtained through the courtesy of Dr. Cake of the Herman Kiefer Hospital. The agglutination tests were made by Marguerite Lambert.

Voldrich have recently reported the presence of agglutinins for Staphylococcus aureus in tuberculous effusions.[10]

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THE PREGNANCY TEST IN RABBITS*

Operative Demonstration of the Ovaries With Minimal Antisepsis

Raymond H. Goodale, M.D., and Mary C. Flanagan, B.S., Worcester, Mass.

RABBITS have entirely replaced mice for the pregnancy test in our laboratories. It is the purpose of this paper to describe our operative procedure with minimal preparation and antisepsis for demonstrating the ovaries. This makes it possible to use one rabbit at least three times. The cost of a test, however, is actually a little more than one-third of the price of a rabbit because of the chemicals and suture material used. The saving is considerable in an institution doing a large number of tests.

With the rabbits under ether anesthesia laparotomies were done at first. We had a high mortality in spite of moderately careful asepsis. This was undoubtedly due to the transabdominal approach to the ovaries, requiring a large incision with a consequent exposure and infection of the peritoneum. Since the ovaries lie against the back of the rabbit at the ends of the uterine horns, the idea of an approach through the back seemed logical. This method has been used successfully in performing oophorectomies on mice.

TECHNIC

The Friedman technic[1] was followed in administering the urine to be tested. Rabbits used were females three to four months old. Occasionally, however, we have used adult females which had been isolated singly for one month. The animals were given 7 to 10 c.c. of a filtered morning specimen

*From the Worcester City Hospital Pathological Laboratory and the Medical Arts Clinical Laboratory.
Received for publication, February 6, 1932.
[1]Friedman, M. H.: Mechanism of Ovulation in Rabbit. Ovulation Produced by Injection of Urine From Pregnant Woman, Am. J. Physiol. **90**: 617-22, 1929.

of urine through the marginal ear vein. Care was taken to make sure that the urine was not below room temperature. We had one fatality after administering urine soon after it was taken from the ice box. A minimal period of thirty-six hours was allowed to elapse before examining the ovaries.

Preparation.—The rabbit, under ether anesthesia, is placed in the position normally assumed by the rabbit when at rest. The lower back is covered with tincture of iodine which is rubbed into the hair with a sponge. The hair was cut with scissors on some rabbits, and it was found easier to suture the skin.

The instruments necessary are a scalpel, tooth forceps, a hook, scissors, needle and needle holder, and a No. 1 catgut suture (Fig. 1). These are placed in a dish of 70 per cent alcohol. The operator wearing rubber gloves rinses

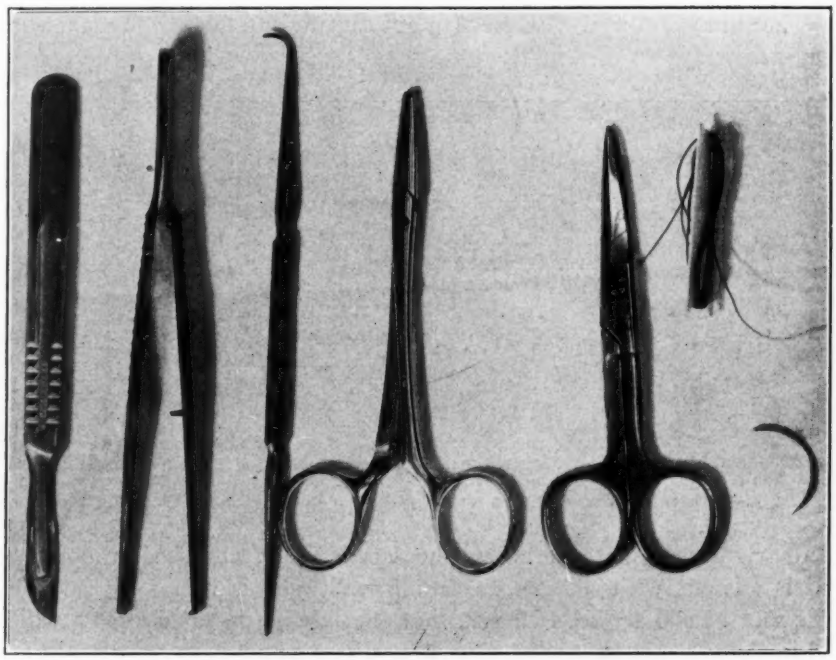

Fig. 1.—Instruments used in operation.

his finger tips in the alcohol. (The alcohol is filtered after the operation and is used again.)

Operative Procedure.—The operator locates the fifth vertebra above the juncture of the femur with the coxal or pelvic bone. A 4 cm. longitudinal incision is made in the midline and should be bisected by the above mentioned vertebra. The incision is carried completely through the skin which is then readily separated from the fascia and muscle layer beneath. Because of the looseness of the rabbit skin the single incision serves for the examination of both ovaries.

The skin incision is now pulled to one side until the white line of the lumbodorsal fascia is seen. This is usually about 2 cm. from the midline of the vertebral column and is just lateral to the thick sacrospinalis muscle. A 2 cm. incision is made through the lumbodorsal fascia. The intestines are then

seen through the parietal peritoneum. The peritoneum is picked up with forceps and is cut allowing entrance into the abdominal cavity. The hook is then inserted and the ovary is brought into view. The ovary is usually found medial to the incision. The hook sometimes engages with the horn of the uterus or with the tube, but in either case the ovary is readily found. In a careful operation bleeding is not usually encountered. The ovary is inspected (Fig. 2), and then dropped back into the peritoneal cavity. The same procedure is repeated on the other side through the one skin incision by drawing it over to the proper position. If the first ovary is definitely positive, the second ovary is not examined.

The incisions in the fascia are not sutured for two reasons: First, because of the difficulty in entering at subsequent operations and second, because of the time involved. The rabbits thus far have not had a hernia through these openings. The skin is sutured with No. 1 plain catgut, and alcohol is soaked into the suture line.

Fig. 2.—Operative wound with ovary exposed on hook. Rabbit in prone position.

In early subsequent operations the catgut is not absorbed, and the healing wound is cut out with scissors. Granulation tissue is found in the old fascia wounds and bleeds slightly. Sometimes the uterine horn or ovary will be involved in the granulation tissue. Intestines have never been found in the granulation tissue.

DISCUSSION

Some operations were performed after using one grain of sodium amytal intravenously. This acts as an effective anesthetic on rabbits, and they remain unconscious for five to six hours. It is not used regularly, however, because of the cost.

In a series of 61 operations on 35 rabbits we have had 5 deaths from peritonitis. Two deaths occurred after three operations, and three occurred after one operation. In general the rabbits cannot be used for more than three tests because of thrombosis of ear veins or granulation tissue in the wounds of the lumbodorsal fascia.

The test has been repeated as early as three days following an operation which revealed negative ovaries. A subsequent paper will deal with the results of repeated tests in both negative and positive rabbits.

The defects in the asepsis are fully recognized. It is, therefore, nothing more than partial antisepsis. As such we have had a low mortality and feel that the procedure is worth continuing. Even with the operative time of five to seven minutes plus the cost of materials there is a definite saving on each test.

An operative procedure on rabbits is helpful when one desires a diagnosis of pregnancy at the end of fifteen hours. The diagnosis can then be confirmed by examining the ovaries of the rabbit again at the end of thirty-six hours, and thus save injecting a second rabbit.

The question arises as to the disposal of rabbits after the third operation. We have exchanged three used rabbits for one fresh rabbit. The dealer is now trying to breed them.

SUMMARY

A simple operative procedure is described for demonstrating rabbits' ovaries. The operation is used primarily in carrying out the pregnancy test in rabbits. The cost of the test is reduced almost two-thirds, and the extensive preparation and asepsis of a laparotomy are not necessary.

The authors wish to express appreciation of the technical assistance of Matthew Carrigan.

BACTERIOLOGIC TESTING OF CATGUT SUTURES*

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IN CARRYING out most bacteriologic examinations, the object to be attained is the demonstration of the presence or the absence of some particular species of bacteria. In the case of surgical sutures, however, the purpose of the bacteriologic tests is to prove the entire absence of all forms of bacteria, absolute sterility. Such a procedure must, of necessity, involve a highly specialized technic carried out under the most rigid conditions.

CONTAMINATED COMMERCIAL CATGUT SUTURES

Competent bacteriologists, after making extensive bacteriologic examinations over a period of years of commercial catgut sutures marketed in various countries, have reported a high percentage of contaminated sutures. Thus, in England, Bulloch[1] found more than 75 per cent of the sutures which he examined of eight different manufacturers to be infected with living bacteria. In Germany, Knorr[2] found that at least 80 per cent of the commercial catgut sutures that he examined contained both pathogenic and nonpathogenic bacteria. In the United States, Meleney and Chatfield[3] examined sutures from seventeen different manufacturers and found that the products of seven manufacturers contained living bacteria.

Because of the large percentage of contaminated commercial catgut sutures reported in various countries by competent bacteriologists, the careful bacteriologic testing of catgut sutures by an approved method and with adequate con-

*Read before the Section on Medical Bacteriology, Immunology and Comparative Pathology at the Thirty-Third Annual Meeting of The Society of American Bacteriologists, Baltimore, Md., December 30, 1931.
Received for publication, January 5, 1932.

trols is of paramount importance to bacteriologists as well as to the surgical profession.

THE PROPOSED STANDARD BACTERIOLOGIC TEST FOR CATGUT

As a result of their three-year study of all existing bacteriologic methods of testing catgut sutures, Meleney and Chatfield devised and proposed what they termed a standard test for effectively determining the sterility of catgut. Some of the essential steps in this technic are similar to those devised six years ago by Dr. Benjamin White, and successfully used by him during the five-year period prior to the time the standard test was proposed. However, this standard test possesses distinct improvements and advantages over the methods previously used for testing catgut.

Anaerobiosis.—The anaerobic culture medium for the standard test is that devised by Novy[4] in 1893 and so successfully used by him since that time for anaerobic culture work. The anaerobic seal that was recommended by Meleney and Chatfield was originally devised by Hall[5] in 1929 in connection with the study of anaerobes. He coined the name "vaspar," and recommended it as a seal which effectively prevents evaporation as well as the reabsorption of oxygen. Hall also found that the white mineral oil, so widely used as a seal, is inefficient because it is permeable for oxygen; and his findings were confirmed by Meleney and Chatfield.

Incubation Period.—For several years, the incubation period recommended and used by Dr. Benjamin White for testing surgical sutures was fourteen days. This period of time had always proved adequate; because it provided ample opportunity for even small numbers of bacteria that might be present in the catgut to adapt themselves to the new environment, and for the culture medium to reach a reduction potential satisfactory for their growth. The Therapeutic Substances (Catgut) Regulations of 1930 adopted by Great Britain specify a period of twelve days for incubating the tubes of culture medium containing the catgut sutures. However, Meleney and Chatfield found that in a large number of the tubes of culture medium containing catgut sutures, the first evidence of growth appeared as late as the twelfth or thirteenth day; and, in order to provide a margin of safety, they recommended an incubation period of fifteen days.

Neutralizing Fluids.—Various chemical substances used in the treatment of the catgut itself or in the tubing fluid, even when present in very high dilution, will often inhibit the growth of bacteria. Hence, they exert a bacteriostatic action. Unless these chemicals are dissolved and removed from the catgut, so that their action is completely neutralized, they will be carried over into the culture medium where they will inhibit growth of bacteria that may be contained within the catgut.

The first to advise the use of neutralizing fluids was Geppert[6] who recommended ammonium sulphide for removing mercurial salts. About six years ago, Bulloch advocated the use of one per cent sodium thiosulphate as a neutralizing solution for metallic salts. Later, he and his coworkers recommended the use of ammonium sulphide, or sodium thiosulphate with sodium carbonate. The British Therapeutic Substances (Catgut) Regulations of 1930 specify one per cent sodium thiosulphate with one per cent sodium carbonate as a neutralizing solu-

tion; and this solution was adopted and recommended by Meleney and Chatfield for removing the chemicals from catgut.

Controls.—Adequate controls are essential if the bacteriologic test is to prove reliable and efficient. Meleney and Chatfield attempted to surround their standard test with every precaution; and the controls which they specified are both important and necessary. However, in addition to their controls, other controls are also required if the test is to be adequately safeguarded. In carrying out the standard test, I use the following three additional controls which I feel are essential: (1) Two or more tubes of culture medium are planted with 10 c.c. of each lot of distilled water and incubated for fifteen days to determine the sterility of the water. (2) At least two tubes of culture medium are planted with 10 c.c. of each lot of the neutralizing fluid and incubated for fifteen days to determine sterility of the solution. (3) Two tubes or more of the culture medium not planted with catgut, but sealed with a layer 2 cm. thick from every batch made of the "vaspar" mixture, are incubated fifteen days to determine sterility of the "vaspar" seal.

During the past fourteen months, I have been using the proposed standard bacteriologic test, with the additional three controls just mentioned, for determining the sterility of surgical catgut sutures. Many thousand lots of sutures have been tested during this time, so that I have had ample opportunity of determining whether or not the test is effective.

CONCLUSIONS

1. Adequate controls are essential for the effective application of the standard bacteriologic test; and three additional controls are herein described and recommended.

2. The standard test for sterility, proposed by Meleney and Chatfield, when used with the three additional and essential controls herein described, and when used in conjunction with suitable neutralizing fluids for dissolving and removing the chemical compound with which the sutures may be impregnated, represents the most efficient and reliable control of the sterility of surgical catgut sutures.

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A MODIFICATION OF TECHNIC IN TERRY'S METHOD OF RAPID TISSUE DIAGNOSIS*

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THE following technic has been found very satisfactory, particularly in the cutting of uterine scrapings for rapid diagnosis. In Terry's original technic a thin slice of the tissue for examination is cut with a razor. The surface of this tissue is then stained with a polychrome methylene blue, the excess stain is washed off and the tissue is examined. While this method can be applied to solid pieces of tissue it is not applicable to uterine scrapings and other small bits of tissue.

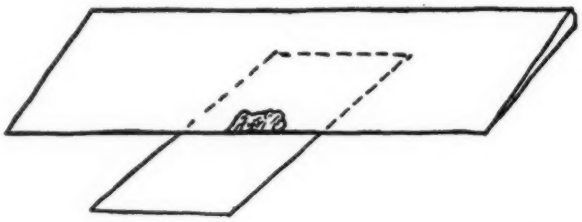

Fig. 1.—Position of slide in reference to knife and tissue.

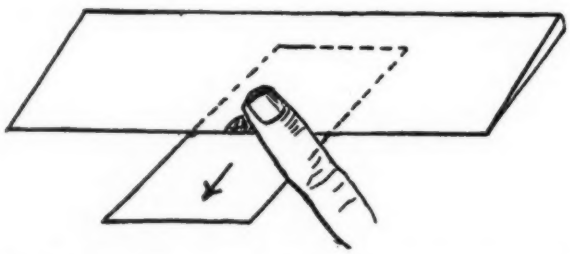

Fig. 2.—The finger and slide are both moved in the direction of the arrow.

The difficulty of cutting small pieces of soft tissue can be overcome by first freezing the pieces and then cutting the frozen mass as follows: The uterine scrapings or other small pieces of tissue are placed on the stage of a freezing microtome and frozen en-masse. While the tissue is still frozen, a section is cut about 1/16 to 1/32 of an inch in thickness. The thick section is prevented from "rolling up" by guiding it with the index finger of the left hand. The frozen section then lies flat on the microtome knife. The frozen section is then quickly transferred to a slide before thawing takes place. This is done by placing a slide perpendicular to the cutting blade of the knife and partially under that portion of the knife on which the section lies (Fig. 1). Then while the left hand slowly draws the slide forward the index finger of the right hand is placed on the partially frozen section and it is also drawn forward (Fig. 2), transferring the section onto the slide. This operation must be done rapidly as it will be found

*Received for publication, February 1, 1932.

very difficult to keep the numerous small pieces of tissue flat and together if the thick section is not partially frozen during the transfer. The section is then stained by placing two or three drops of Terry's stain on the tissue. The slide and section are then immersed in water, the slide being held horizontal during this process and the immersion being carried out very slowly as any currents of water are liable to dislodge the pieces of tissue. When the proper amount of stain is washed off, which will be a matter of a few seconds, the slide is removed from the water, being still held in an even position to prevent the tissue from being washed off. The excess water and stain are then wiped off the slide and the cover slip is placed on the stained tissue. It is then ready for microscopic examination.

1016 Delaware Avenue.

A COLLOIDAL CARBON FLOCCULATION TEST IN SPINAL FLUID*

Preliminary Report

Purcell G. Schube, M.D., Hartford, Conn., and Herbert E. Harms, Denver, Colo.

ALTHOUGH there have been advocated a number of methods with varying technics in attempting a fractional analysis of spinal fluid, all possess defects which improvement and refinement fail to remove. However, with the introduction of each of these there has been given to the field of the chemistry of spinal fluid an additative or eliminative contribution. These contributions serve two purposes: first, they add to or subtract from the diagnostic facilities of the laboratory; and second, they advance, in every instance, the understanding of the mechanisms underlying the pathology of the fluids.

It is because of these that we feel justified in presenting a preliminary report of a study in colloidal chemistry of a flocculation reaction of colloidal carbon in a series of spinal fluids. The theoretical aspects of the underlying chemical principles will be presented in a later and more extensive report of the work. Herein are tabulated the laboratory results with comparative tests which are routinely performed upon all spinal fluids in this institution.

METHOD OF SECURING SPINAL FLUID

All cases admitted to the Colorado Psychopathic Hospital receive admission lumbar punctures from which fluid is drawn into sterile tubes for total protein, sugar, Wassermann and colloidal gold tests. Part of this fluid was used for the colloidal carbon flocculation test. Each fluid was set up in duplicate, in the later set.

The fluid for the colloidal carbon flocculation test was placed in an ice box until used. The length of time during which the fluid stood did not affect the test as long as the fluid remained sterile. All contaminated fluids were discarded.

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*From the University of Colorado Psychopathic Hospital, and Medical School.
Received for publication, February 4, 1932.

METHOD OF PREPARATION OF TEST

Seven tubes which had been washed thoroughly, rinsed with distilled water and dried were used. For convenience, test tubes of 100 mm. by 13 mm. and of 125 mm. by 16 mm. were used. The size or caliber of the tube used did not affect the test. These were placed in a rack and numbered 1, 2, 3, etc., through 7.

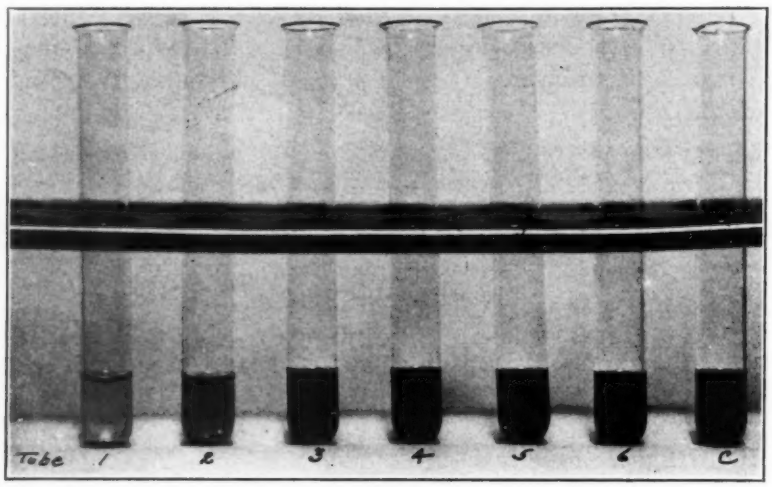

Fig. 1.

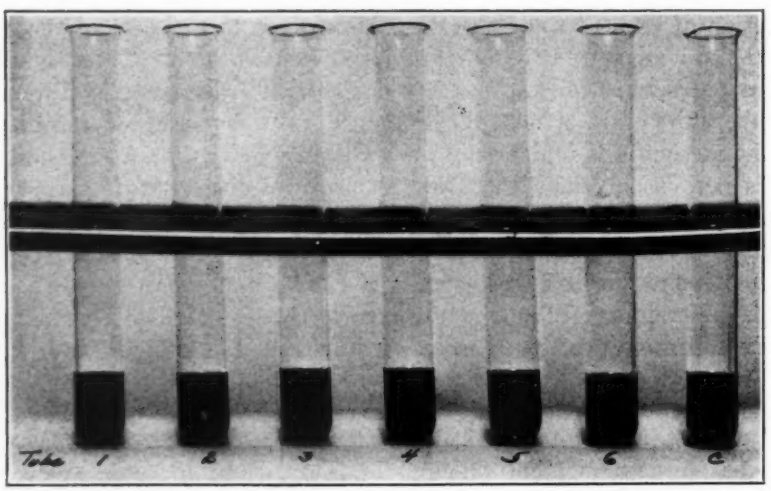

Fig. 2.

In each tube was placed 1 c.c. of distilled water. In tube No. 1 was placed 1 c.c. of spinal fluid. The contents of tube No. 1 were thoroughly mixed and 1 c.c. of this mixture was transferred to tube No. 2. This process was repeated through tube No. 6, the last 1 c.c. being discarded. Tube No. 7 acted as the control.

In each of the seven tubes was then placed 0.1 c.c. of 0.1 per cent oxalic acid solution. Each tube was again thoroughly mixed. In each tube was

then added 0.4 c.c. of 1 per cent colloidal carbon. (The 1 per cent colloidal carbon was made by diluting 1 c.c. of Carter's Black India Ink No. 358 to a total volume of 100 c.c. with distilled water at room temperature.) Each tube was finally agitated until thoroughly mixed.

The tubes were then set aside at room temperature and read twelve hours later.

METHOD OF READING TUBES

The tubes were read, dependent upon the result obtained, as either "flocculation" or "no flocculation." The "flocculation" was a definite aggregation of the colloidal carbon particles which settled to the bottom of the tube leaving a clear, supernatant fluid. The "no flocculation" was a stable suspension of the colloidal carbon particles and tube No. 7 served as this control. Any questionable degree of murkiness between these two extremes was considered as "no flocculation."

Fig. 1 is a typical "flocculation" set. Fig. 2 is a typical "no flocculation" set

The "flocculation" was designated by the letter "P." The "no flocculation" was recorded as "O."

RESULTS

Tables I and II, are the findings in the first 100 cases upon which the test was used.

TABLE I

DIAGNOSIS	NUMBER OF CASES
General paresis	26
Schizophrenia (Dementia precox)	20
Senile psychosis	7
Organic brain disease	6
Epilepsy (idiopathic)	6
Alcoholism	6
Manic-depressive psychosis, depressed phase	5
Cerebro-arteriosclerosis	3
No psychosis	2
Manic-depressive psychosis, manic phase	2
Taboparesis	2
Meningovascular syphilis	2
Psychoneurosis	2
Posttraumatic constitution	2
Manic-depressive psychosis	1
Hydrocephalus	1
Diabetes insipidus	1
Multiple sclerosis	1
Cerebral hemorrhage	1
Constitutional psychopathic inferiority	1
Mental defective	1
Toxic psychosis	1
Korsakow's psychosis	1
Total	100

SUMMARY

1. The method of conducting a colloidal carbon flocculation test in spinal fluid is described.

2. The results obtained with this test in 100 consecutive cases are presented

TABLE II

CASE NUMBER	DIAGNOSIS	AGE	BLOOD WASSERMANN	SPINAL FLUID WASSERMANN	SPINAL FLUID PROTEIN	SPINAL FLUID SUGAR	GOLD CURVE	COLLOIDAL CURVE	TREATMENT	PREVIOUS WORK		
										BLOOD WASSERMANN	SPINAL FLUID WASSERMANN	GOLD CURVE
1	General Paresis	55	0-0	4-1	130	39	5555544320	PP00000				
2	General Paresis	52	0-0	0-0	10	50	1113310000	P000000	T	0-2	4-4	3334555540
3	General Paresis	38	4-4	4-4	130	53	5555544430	PPP0000				
4	General Paresis	38	4-4	4-4	35	62	5555410000	PP00000				
5	General Paresis	47	0-0	4-4	20	50	5554432000	PP00000				
6	General Paresis	56	0-0	0-0	25	72	0000000000	P000000	T	0-0	4-4	4444555100
7	General Paresis	38	4-4	0-0	18	77	0112210000	PP00000				
8	General Paresis	20	0-0	0-0	50	80	2223321000	PP00000				
9	General Paresis	45	4-4	4-4	70	37	5555443100	PP00000				
10	General Paresis	39	0-2	4-4	40	90	5554443200	PP00000				
11	General Paresis	44	0-0	3-4	30	54	2223200000	PP00000	T	0-0	3-4	2223200000
12	General Paresis	41	0-0	0-0	70	53	2223321000	P000000	T	4-4	4-4	3333455500
13	General Paresis	39	0-0	0-0	38	103	5544310000	P000000	T	0-0	1-4	1111220000
14	General Paresis	35	0-0	1-4	30	74	2223443000	P000000	T	3-3	4-4	2233455440
15	General Paresis	42	4-4	0-0	15	78	0000000000	PP00000	T	—	4-4	1555442100
16	General Paresis	45	0-0	0-0	55	81	0001110000	P000000	T	—	0-4	4455555550
17	General Paresis	31	4-4	4-4	50	81	5554321000	PP00000	T	4-4	4-4	5555421000
18	General Paresis	36	—	3-2	20	58	5555444310	PP00000	T	—	4-4	5555431000
19	General Paresis	40	—	0-0	28	50	0011310000	P000000	T	0-0	—	0112210000
20	General Paresis	58	—	0-0	33	72	0000000000	PP00000	T	4-4	4-4	3344445550
21	General Paresis	51	—	4-4	75	66	5555443210	PP00000	T	0-0	4-4	5544322000
22	General Paresis	33	2-4	4-4	80	64	5555554440	PP00000				
23	General Paresis	59	4-4	4-4	105	77	5555544420	PP00000				
24	General Paresis	60	0-4	4-4	155	50	5555543320	PP00000				
25	General Paresis	45	—	4-4	70	53	5555543200	PP00000				
26	General Paresis	19	4-4	4-4	100	72	5555544430	PP00000				
27	Tabo-Paresis	52	4-4	4-4	60	98	0011222100	PP00000				
28	Tabo-Paresis	54	0-0	0-0	70	86	0011210000	P000000	T	—	3-4	2112100000
29	Meningo-Vascular Syphilis	26	—	4-4	75	69	5555544110	PP00000				
30	Meningo-Vascular Syphilis	27	0-0	4-4	40	74	2223321000	PP00000				
31	Multiple Sclerosis	35	0-0	0-0	40	76	5555544110	0000000				
32	Schizophrenia	44	0-0	0-0	55	75	0000000000	0000000				
33	Schizophrenia	26	0-0	0-0	23	48	0000000000	0000000				
34	Schizophrenia	35	0-0	0-0	23	90	0000000000	0000000				
35	Schizophrenia	25	0-0	0-0	60	91	0011100000	0000000				
36	Schizophrenia	40	0-0	0-0	15	58	0011000000	0000000				
37	Schizophrenia	38	0-0	0-0	20	66	0000000000	0000000				
38	Schizophrenia	44	0-0	0-0	20	50	0000000000	0000000				
39	Schizophrenia	53	0-0	0-0	50	85	0001100000	0000000				
40	Schizophrenia	40	0-0	0-0	35	81	0000000000	0000000				
41	Schizophrenia	52	0-0	0-0	33	90	0000000000	0000000				
42	Schizophrenia	19	0-0	0-0	55	68	0000000000	0000000				
43	Schizophrenia	42	0-0	0-0	43	55	0001100000	0000000				
44	Schizophrenia	39	0-0	0-0	25	90	0000000000	0000000				
45	Schizophrenia	28	0-0	0-0	30	94	0000000000	0000000				
46	Schizophrenia	49	0-0	0-0	55	83	0000111000	0000000				
47	Schizophrenia	22	0-0	0-0	33	85	0000000000	0000000				
48	Schizophrenia	20	0-0	0-0	50	66	0001110000	0000000				
49	Schizophrenia	30	0-0	0-0	35	67	0012100000	0000000				
50	Schizophrenia	20	0-0	0-0	15	60	0000000000	0000000				
51	Schizophrenia	25	0-0	0-0	60	91	0001110000	0000000				
52	Senile Psychosis	55	0-0	0-0	30	40	0000000000	0000000				
53	Senile Psychosis	86	0-0	0-0	32	73	0001110000	0000000				
54	Senile Psychosis	66	0-0	0-0	58	92	0000000000	0000000				
55	Senile Psychosis	59	0-0	0-0	73	69	0001110000	0000000				
56	Senile Depression	45	0-0	0-0	20	69	0000000000	0000000				

TABLE II (CONTINUED)

CASE NUMBER	DIAGNOSIS	AGE	BLOOD WASSERMANN	SPINAL FLUID WASSERMANN	SPINAL FLUID PROTEIN	SPINAL FLUID SUGAR	GOLD CURVE	COLLOIDAL CURVE	TREATMENT	PREVIOUS WORK			
										BLOOD WASSERMANN	SPINAL FLUID WASSERMANN	GOLD CURVE	
57	Senile Deterioration	69	0-0	0-0	50	75	0000000000	0000000					
58	Senile Psychosis	64	0-0	0-0	43	82	0012200000	0000000					
59	Organic Brain Disease	35	0-0	0-0	50	72	0000000000	0000000					
60	Organic Brain Disease	41	0-0	0-0	60	27	0000111000	0000000					
61	Organic Brain Disease	27	0-0	0-0	28	61	0000000000	0000000					
62	Organic Brain Disease	52	0-0	0-0	45	104	1112100000	0000000					
63	Organic Brain Disease	48	0-0	0-0	70	63	0001110000	0000000					
64	Organic Brain Disease	45	1-3	0-0	35	70	0001110000	0000000					
65	Epilepsy	23	0-0	0-0	15	81	0000000000	0000000					
66	Epilepsy	13	0-0	0-0	20	70	0011100000	0000000					
67	Epilepsy	51	0-0	0-0	45	70	0012100000	0000000					
68	Epilepsy	11	0-0	0-0	35	65	0000000000	0000000					
69	Epilepsy	2	0-0	0-0	35	72	0000000000	0000000					
70	Epilepsy	17	0-0	0-0	43	66	0001100000	0000000					
71	Alcoholism	36	0-0	0-0	40	96	0000000000	0000000					
72	Alcoholism	40	0-0	0-0	15	75	0000000000	0000000					
73	Alcoholism	41	0-0	0-0	25	93	0000000000	0000000					
74	Alcoholism	24	0-0	0-0	15	64	0000000000	0000000					
75	Alcoholism	38	0-0	0-0	43	58	0000000000	0000000					
76	Alcoholism	34	0-0	0-0	25	—	—	0000000					
77	Depression	33	0-0	0-0	18	59	0001110000	0000000					
78	Depression	20	0-0	0-0	25	57	0000000000	0000000					
79	Depression	37	0-0	0-0	60	69	0000000000	0000000					
80	Depression	41	0-0	0-0	25	96	0000000000	0000000					
81	Depression	53	4-4	0-0	20	75	0000000000	0000000					
82	Arteriosclerosis	68	0-0	0-0	25	50	0000000000	0000000					
83	Arteriosclerosis	82	0-0	0-0	28	98	0000000000	0000000					
84	Arteriosclerosis	62	0-0	0-0	20	80	0000000000	0000000					
85	No psychosis	45	0-0	0-0	40	85	0000000000	0000000					
86	No psychosis	39	0-0	0-0	15	73	0000000000	0000000					
87	Manic with Paranoid Trends	47	0-0	0-0	50	72	0000000000	0000000					
88	Manic Psychosis	24	0-0	0-0	28	72	0000100000	0000000					
89	Psychoneurosis	21	0-0	0-0	25	63	0000000000	0000000					
90	Psychoneurosis	45	0-0	0-0	70	117	0001110000	0000000					
91	Post Traumatic Constitution	36	0-0	0-0	28	95	0001100000	0000000					
92	Post Traumatic Epilepsy	31	0-0	0-0	28	58	0011100000	0000000					
93	Mental Defective	28	0-0	0-0	70	74	0000000000	0000000					
94	Cerebral Hemorrhage	35	0-0	0-0	28	67	0000000000	0000000					
95	Toxic Psychosis	34	0-0	0-0	15	91	0001100000	0000000					
96	Manic-Depressive Psychosis	29	0-0	0-0	30	77	0000000000	0000000					
97	Diabetes Insipidus	14	0-0	0-0	25	54	0001100000	0000000					
98	Hydrocephalus	2	0-0	0-0	66	25	0011000000	0000000					
99	Psychopathic Inferiority	28	0-0	0-0	20	121	0000000000	0000000					
100	Korsakoff's Psychosis	41	0-0	0-0	25	96	0000000000	0000000					

with the comparative laboratory work—gold curve, Wassermann (blood and spinal fluid), spinal fluid protein and spinal fluid sugar.

3. There appears to be a definite correlation between this test and neurosyphilis.

The authors wish to express their appreciation for the constructive criticism of the work by Drs. George S. Johnson, Edward R. Mugrage, and Robert C. Lewis.

AN AUTOMATIC PIPETTE FOR THE ACCURATE DELIVERY OF VARIABLE QUANTITIES OF LIQUIDS*

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VARIOUS laboratory procedures, such as the examination of water, milk, and sewage for total bacterial count, require the use of dilution flasks containing exact quantities of sterile water. Certain other procedures require the use of large numbers of tubes containing known quantities of culture media. Since the distribution by hand of these liquids is very time consuming, several pipettes have been designed to automatically measure and distribute this material. In general, these appliances, which are usually made in the form of a four-way stopcock so connected to two side arms that one of these arms is filling while the other is emptying, have a serious disadvantage in that they deliver only a fixed quantity of fluid, and any losses in the amounts delivered, due to subsequent sterilization, can be remedied only by the direct addition with a pipette of fresh liquid to the individual containers. Furthermore, since these devices are constructed of glass, they are easily broken, necessitating care in their handling.

Since the amount of liquid lost from a container during the process of sterilization varies with the composition, size, and shape of the container, the amount and nature of the fluid contained therein, and the peculiarities of the particular autoclave in use, the use of a delivery pipette of fixed capacity for the distribution of liquids is often very undesirable. To be satisfactory for general laboratory use, an automatic pipette should not only be unbreakable, but should be so constructed that it can be rapidly and easily adjusted to any desired capacity.

Requiring in our laboratory numerous tubes of culture media, the volume of which after sterilization had to be kept within extremely close limits, we endeavored to design a delivery device so constructed as to overcome the disadvantages inherent in existing types of automatic delivery pipettes. The details of construction of this apparatus, which has been designed throughout with the object of speed and accuracy in use, are given below.†

The outer section or shell of the apparatus consists of a four-way stopcock, constructed of brass or bronze stock, from the two sides and front of which project side arms, as shown in Figs. 1, 2, and 3. All external parts of the apparatus

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Received for publication, January 29, 1932.
†This apparatus may be obtained from the Arthur H. Thomas Company, Philadelphia.

are thoroughly polished so as to aid in cleaning the device. For the sake of strength and appearance, the top of the shell terminates in a heavy shoulder.

A somewhat smaller side arm projects from the rear of the shell to which is fastened an inlet tube for the liquid being distributed. Should it prove difficult to make the apparatus in one piece similar to the manufactured article, the side arms may be separately turned from brass or bronze stock, and fastened to the main shell by means of properly made soldered joints.

With the aid of a lathe, the shell is carefully hollowed out, leaving an opening extending completely through the apparatus. The walls of this opening converge inward from top to bottom at an angle of about 3.2°, and must be carefully finished to fit the inner, movable portion of the stopcock, to prevent the accidental

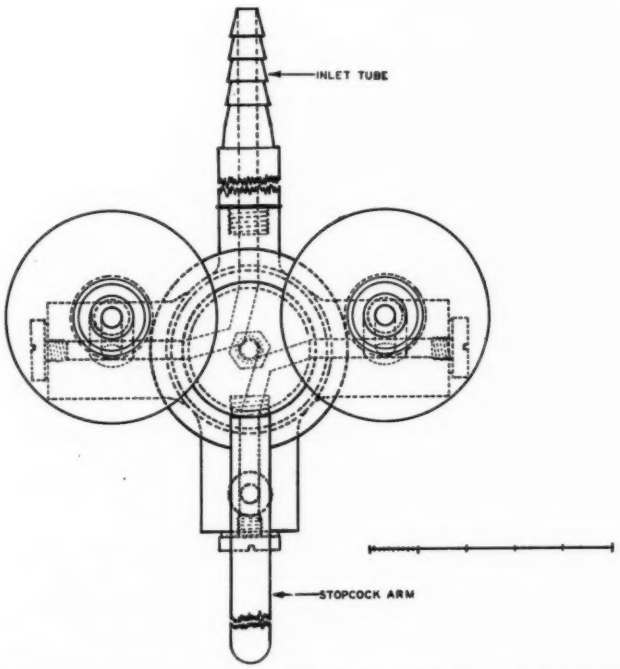

Fig. 1.—Top view of automatic test tube filler. (Main divisions of scale indicate centimeters.)

escape of liquid from the device. The shell walls should be 3 to 4 mm. in thickness.

The inner movable portion of the stopcock is constructed of brass or bronze stock, carefully machined so that when ground into position, the top projects approximately 12 mm. above the shoulder of the outer shell when placed in position, as shown in Fig. 2. A handle of any desirable length and diameter is fitted into the projecting portion of this center piece by means of a threaded or soldered joint.

Holes 4 mm. in diameter are drilled through the front, back and side arms of the outer shell, at angles exactly 90° to each other, as shown in Fig. 1. The movable portion of the stopcock is then drilled as shown in Fig. 1, so that the various openings exactly coincide with the holes in the outer shell. The holes

must accurately coincide, whether the movable arm is turned straight forward, or 90° to either side of the forward position.

The inlet tube, which is made so as to tightly hold a rubber tube, is fastened to the rear arm of the shell by means of threaded connections. The bore of this tube should be 4 mm. in diameter.

An outlet tube, the bore of which is 4 mm. in diameter, is connected to the front arm of the shell by means of a soldered joint. The tip of the outlet tube is sharply bevelled, to insure accuracy of delivery of the fluid.

Two side arm uprights, made of heavy wall brass tubing of 4 mm. internal diameter, are constructed as shown in Figs. 2 and 3. These uprights are coarsely threaded for all but a short portion of their lower length so that movable brass

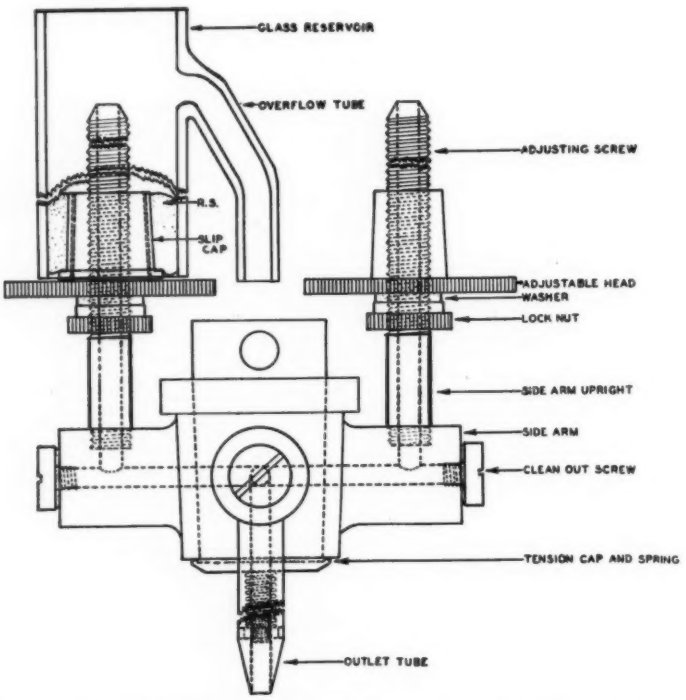

Fig. 2.—Top view of test tube filler, showing one glass reservoir in place, and the method of attaching the reservoirs to the adjustable heads.

platforms, tapped to fit the threads of the tubing, can be adjusted to occupy any position on the tubes. These adjustable heads or movable platforms can be locked in position at any point by means of leather washers and brass lock nuts, as shown in the diagrams. The threaded portions of the tubes should be of such a length that the adjustable heads have a range of motion of at least 9 to 10 cm. The upper ends of the uprights are carefully bevelled so as to produce sharply defined menisci, thus increasing the accuracy of volume of the various portions of liquid withdrawn from the outlet tube.

The adjustable heads consist of large flat platforms, the upper sides of which terminate in cones machined so that tapered brass shells or caps can be placed in position over them and seated by means of a twisting motion so as to produce a

water tight seal. These caps should be fitted so as to rotate freely without binding.

Glass reservoir tubes of various capacities are made, to be attached to the tapered slip caps by means of rubber or wax seals. Each glass reservoir has an overflow tube so located that, with the adjustable head about 2 cm. from its highest possible position the maximum desired volume of liquid is retained within the reservoir when the stopcock is closed. The additional 2 cm. of threading permits further increasing the capacity of the reservoirs to compensate for any losses due to evaporation during sterilization. The capacity of the reservoirs can be adjusted within rather wide limits for larger or smaller amounts of fluid by means of the adjustable heads.

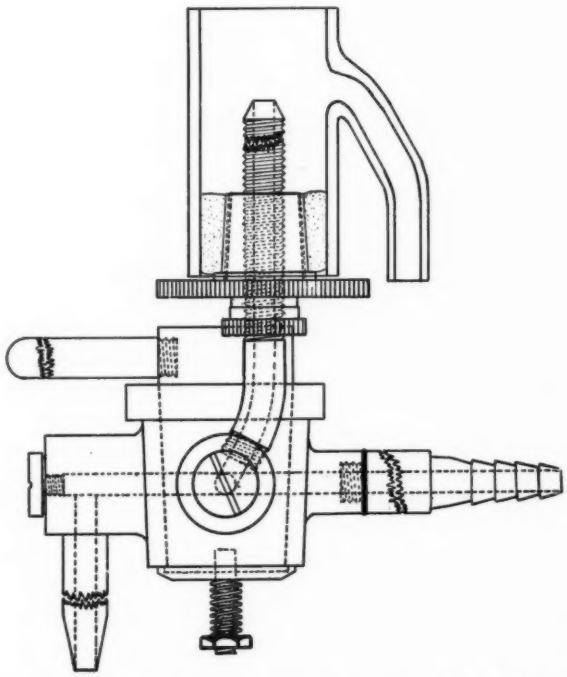

Fig. 3.—Side view of test tube filler, showing details of construction of the spring tension device.

When the apparatus is in use, the two glass overflow tubes are connected together by means of a Y joint, and the excess liquid collected in a container placed at the rear of the apparatus.

The two uprights are fastened to the side arms by means of tightly fitting threaded joints. The side arms are drilled and tapped so that when the upright tubes are fitted and before they are bent into their final position, they point slightly to the rear, rather than straight up and down. Then, with the stopcock arm turned 90° either to the left or the right hand side position, so that the various holes are in accurate alignment, the uprights are bent as shown in Fig. 3 until their outer walls just touch the movable arm, thus acting as stops for the latter.

The opening in the ends of the side and front arms are closed by means of

screws and washers. The apparatus can be thoroughly cleaned by removing these screws and the center movable arm.

Constant and even contact between the center and outer portions of the stopcock is assured by means of a spring tension device, the details of which are shown in Fig. 3.

Rapid and very accurate delivery of any desired amount of liquid into test tubes or flasks is possible by the use of this apparatus. Various sized reservoirs can be used interchangeably, so that by their use it is possible to secure any volume of delivery merely by varying the height of the reservoirs with the aid of the adjustable heads.

A SIMPLE PHOTOGRAPHIC KYMOGRAPH AND TIME RECORDING METHOD*

E. W. Gray, M.D., Thiells, N. Y.

METHODS of employing the smoked paper kymograph for recording various physiologic processes are quite cumbersome, especially where records are to be made on a fairly rapidly moving drum. A few of the difficulties may be summed up briefly as follows:

The writing points are frequently either too tight and refuse to move or are too loose and do not write at all. It is quite necessary that the surface of the kymograph drum and the arc of movement of the writing levers be parallel, otherwise the levers either leave the surface or become too tight when they move. Another disadvantage is the lag caused by friction between the points and the smoked paper. It is scarcely necessary to mention the untidiness brought about by the use of a shellac fixing solution and by smoking the paper.

The use of inked writing points has some of the above disadvantages, and in addition, the ink sometimes stops flowing in the middle of an experiment. The photographic method of recording various physiologic processes is by no means new. The electrocardiograph and photographic polygraph have fully demonstrated this principle. Such apparatus is, however, very expensive.

In order to overcome the latter difficulty, we constructed a simple photographic casing in our laboratory to fit a standard make kymograph. This consists of a square box split vertically in the center to fit around the kymograph drum. The box is constructed of wood except for the front which is a strong sheet of metal with a central vertical slit which when in place lies very close to the surface of the drum. A sliding shutter closes the slit to light and is held in place by a trigger. The shutter (see Fig. 1) opens automatically by the tripping of the trigger when a side arm (see Fig. 2) attached to the shaft of the kymograph strikes one arm of a forked lever. After one complete revolution the side arm strikes the second arm of forked lever (see Fig. 3) and releasing the opening spring allows a weaker (closing) spring to close the shut-

*From the Research Department, Letchworth Village, New York State School for Feebleminded.

Received for publication, December 14, 1931.

ter. In this manner the shutter can be regulated to avoid double exposure of the sensitive paper or film.

Various timing devices may be used depending on the speed and accuracy desired. A timing method for a slowly moving drum has been described by Chillingworth[1] in which the shaft of a synchronous electric clock made a contact once a second thereby energizing a signal magnet. We are employing, in reac-

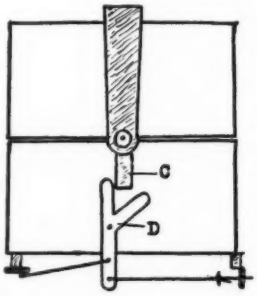

FIG. 2. –
C. Side arm.
D. Forked lever.

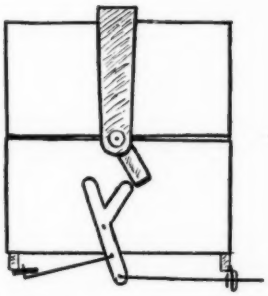

FIG. 3.

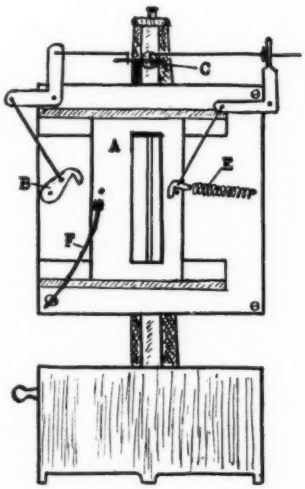

FIG. 1. –
A. Shutter.
B. Trigger.
C. Side arm.
E. Opening spring.
F. Closing spring.

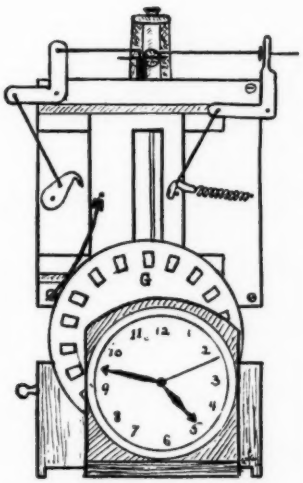

FIG. 4. –
G. Timing disc.

tion time experiments, an aluminum disc with perforations near the edge. The disc is turned by means of a synchronous electric clock exactly one revolution a second. The number of perforations in the circumference determines the number of light flashes that pass through the lower end of the slit per second. The disc we use in most of our work has twenty perforations, and thus records intervals of a twentieth of a second.

A strong beam of light is thrown through the slit. This is accomplished by means of a parallel ray electric light or sunlight reflected from a mirror. Sunlight gives the best records from a photographic viewpoint. We use rapid black bromide paper which comes in rolls twenty inches in width and can be cut as desired. A record taken in our laboratory is reproduced.

The instrument is quite applicable to such experiments as latent period of muscle, velocity of nerve impulse, reflex time, reaction time and, in fact, any experiment where a short paper kymograph is used.

We are employing the above described apparatus to determine simple reaction time to light in a series of mentally deficient children. The reaction time is recorded by means of two signal magnets placed in front of the slit. The lower signal magnet records on the paper the exact moment that a small 3-volt

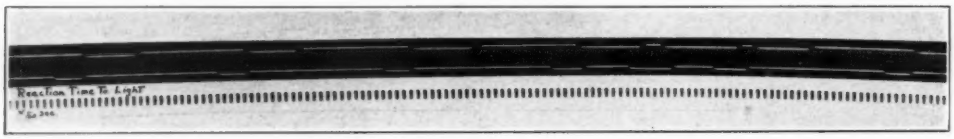

Fig. 5.—Reaction time record reduced to one-third actual size.

light bulb is illuminated. The subject responds by the touch of a light key which activates the upper signal magnet. In this manner not only is the time interval between stimulus and reaction recorded but the duration of the stimulus and the response and also the time interval between stimuli. Another advantage in closing the circuit in both signals is that the lag of each magnet will be practically the same.

SUMMARY

1. A very inexpensive photographic attachment has been developed for a standard make kymograph.

2. A simple and accurate method is described for recording short time intervals photographically.

3. A reduced photograph is shown of a reaction time record made with the apparatus described.

REFERENCE

1. Chillingworth, Felix P.: A New Time Marker, J. Lab. & Clin. Med. **16**: 912, 1931.

A SIMPLE APPARATUS FOR REMOVING GASTRIC CONTENTS FROM DOGS*

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Omaha, Nebr.

IN ORDER to obtain samples of gastric juice from dogs, we have devised a rather simple apparatus, which, in our hands, has yielded very satisfactory results. Briefly, the complete outfit consists of a box to hold the dog and a suction apparatus connected to a stomach tube to obtain the gastric samples.

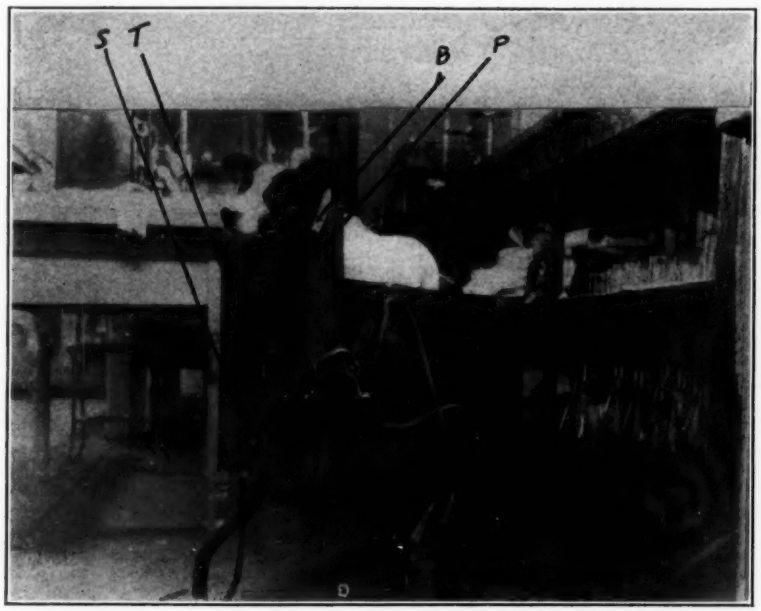

Fig. 1.—B, Chain ensheathed in rubber tubing around dog's neck. P, Post in which front partition is inserted. S, Spindle which holds front partition at desired height. T, Front partition which slides in grooves in P.

The box is so arranged that it can be adjusted to the length and height of the animal used by means of two sliding partitions, one in front sliding up and down, having a U-shaped section cut out at the top (Fig. 1) in order to accommodate the neck of the dog, and the other in back (*G*, Fig. 2) which can be pushed forward or backward, to accommodate the length of the animal. Attached to the front partition is a chain, ensheathed in a small piece of rubber tubing (*B*, Fig. 1), which fits over the dog's neck and thus prevents him from slipping away from the experimenter.

A bit, consisting of a piece of soft wood about six inches long, an inch and a half wide and three-quarters of an inch thick, with a hole sufficiently large to

*From the Department of Physiology, Creighton University School of Medicine.
Received for publication, March 5, 1932.

allow easy entrance of the stomach tube is placed in the animal's mouth when ready for pumping.

The box is thirty inches long, fourteen inches high and seven inches wide and is mounted on four legs. At intervals along the sides slits are drilled through which straps can be passed in order to secure the animal and prevent any undesirable movements (*I*, Fig. 2). These straps can be passed both under and over the animal. We have never found it necessary to strap in our animals, the neck strap and the narrowness of the box being sufficient to prevent any serious disturbances at any time.

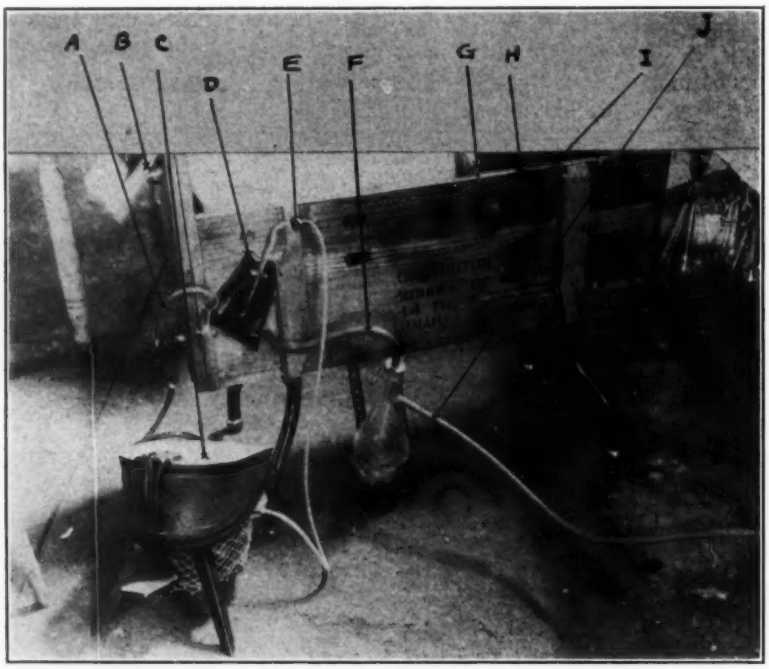

Fig. 2.—A, Stomach tube attached to one end of T-shaped glass tubing. B, Same as in Fig. 1. C, Bellows. D, Wood block in which T-shaped glass tubing is sunken for protection against injury and to render secure. E, Two-way stopcock. F, Heavy rubber tubing connecting lower end of T-shaped glass tubing to suction flask. G, Rear partition which slides forward or backward. H, Grooves which fix rear partition at desired place. I, Slits through which straps may be passed to keep animal steady. J, Heavy rubber tubing connecting suction flask to one of the glass tubes in rubber cork of five-gallon bottle.

Along the side of the box a T-shaped piece of glass tubing is sunk in wooden grooves (*D*, Fig. 2) to prevent its accidental breakage. This glass tubing is the essential feature of the apparatus.

As can be seen from Fig. 2, one end of this T-shaped glass tubing is connected to a pair of bellows through a two-way stopcock (*E*). The other two ends of the T-shaped glass tubing are connected to a suction flask and to the stomach tube respectively. The suction flask is in turn connected by means of heavy rubber tubing (*J*, Fig. 2) to one of two pieces of glass tubing in the rubber cork of a five gallon bottle (not shown in the figures). The other piece of glass tubing in this rubber cork is connected through heavy rubber tubing to the water suction pump. The five gallon bottle is put between the suction pump and the suction flask to prevent the contents of the suction flask from being

pulled into the water pump when the suction is turned off. It has been found advisable to partly fill the bottle with water before using.

When running an experiment the animal is placed in the box, the rear partition adjusted to his length and the front to his height, his neck is pulled forward, the chain passed over it and attached to a hook on the front partition. His mouth is opened and the bit placed transversely across his teeth. The stomach tube is then dipped into water to lubricate it so that it passes easily down the esophagus, inserted into the hole in the bit and passed down into the stomach. Suction is then turned on and within a few seconds the stomach contents will pass into the suction flask. Very often the suction causes the tube to adhere to the stomach mucosa or the tube becomes plugged with mucus or food particles. At this point we resort to the bellows. The stopcock (*E*, Fig. 2) is turned so that air can be passed into the stomach, and the rubber tube connecting the side arm of the glass T-tube with the suction flask is compressed to prevent the air from getting into the suction flask and facilitate its passage into the stomach. When the stomach tube can be freed from the mucosa (determined by attempting to move it about), the stopcock *E* is turned, cutting off the bellows, the rubber tubing *F* is released and the suction is again free to act.

If fluids are put into the stomach by means of a stomach tube an almost quantitative return can be obtained in a few seconds.

We have found that after the first few experiments our dogs rapidly become accustomed to the sensation of a tube being passed and in a very short time offer no resistance.

AN IMPROVED MARKING FOR PRECISION SYRINGES

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THE familiar so-called tuberculin syringe has found probably its greatest utilization in allergic diagnosis and therapy. The allergist very often has occasion to recommend certain dosages of treatment extracts which are to be given either by the family physician or by the patient himself. The presence on all hypodermic syringes of two different scales, the metric and the minim, frequently leads to confusion. No matter how careful one may be in stressing the fact that dosages should be given on the basis of the metric scale, too often the physician or the patient or the nurse uses the minim scale, thereby giving the incorrect dose.

As an example, a patient was being treated by his family physician and was supposed to be getting 0.6 c.c. of 2 per cent ragweed extract. Both the patient and the physician assured me that this was what he was receiving. Reporting to the office for one of his treatments, he was given 0.6 c.c. of 2 per cent ragweed extract which was promptly followed by a mild general anaphylactic reaction. Investigation in collaboration with the physician then developed that after all the patient had been receiving 6 minims instead of 0.6 c.c. When he did receive 0.6 c.c. he was therefore given a 50 per cent larger dose.

The administration of insulin, tuberculin, vaccines, particularly arthritis vaccines, and all other substances in which minute, carefully measured doses must

be given is fraught with this same potential source of inaccuracy as long as there are two different scales on the syringe and when more than one person may be administering the treatment.

To circumvent this disadvantage Becton, Dickinson and Company have collaborated with me in the manufacture of a precision syringe of the tuberculin syringe type, possessing the following advantages:

1. Only the metric scale is used, with graduation to hundredths of a cubic centimeter.

2. In place of the minim scale there is a coarser graduation in tenths and twentieths of a cubic centimeter.

Vaughan Scale.

Vaughan Syringe.

3. The white line running the length of the barrel, between the two scales is omitted, thus providing an observation channel through which the position of the tip of the plunger may be observed.

4. The etching of the gradations on the ordinary tuberculin syringe, while narrow, are still wide enough to cause some small variation in the accuracy of the dosage. The width of the etching on the new syringe, being only five-eighths that of the old, increases precision.

5. In the old style tuberculin syringe when the plunger is pushed way in, its tip does not always correspond exactly to the zero on the scale. In this case, one must be careful in giving hypodermics not to drive the plunger home but to press it just to the zero mark. The new syringe has the zero gradation as accurately as possible on a level with the tip of the plunger when fully inserted.

NOTE ON A METHOD FOR THE MANIPULATION OF BLOOD-DILUTION AND MICRO-PIPETTES*

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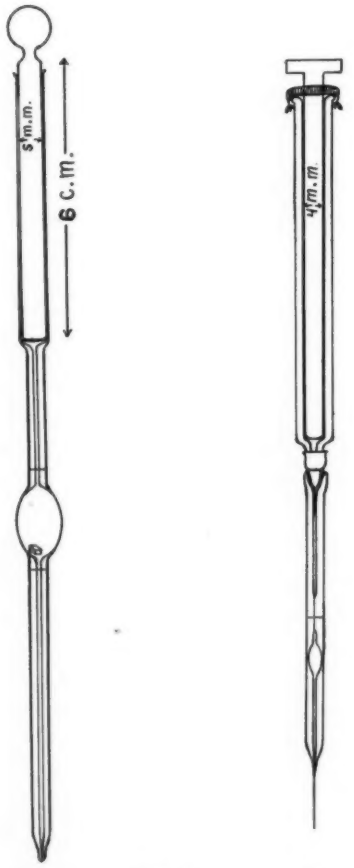
IT IS a well known fact that the manipulation of pipettes for dilution of blood for corpuscle counts is both awkward and liable to be inaccurate due to the difficulty of setting the meniscus accurately on the calibration marks. Inaccuracies of several percentage may be introduced from this source alone unless great care is exercised, and if this is done, the loss of time is considerable. For these reasons, we have constructed the blood-dilution pipette shown in Fig. 1, *A*. The diagram illustrates the design when a dilution of 1 to 10 is desired as in counting the white cells. A sort of syringe was constructed by grinding a plunger into a tube of 5 mm. inside diameter, and this was sealed to the top of a blood-dilution pipette. The device is operated entirely with one hand, thus making it entirely practical for the medical profession, since the ear or finger from which the blood is taken is normally held with the other. The top of the plunger is readily handled with the thumb and forefinger, while the body of the pipette is grasped between the other fingers and the palm of the hand. The plunger is lubricated with a little oil or vaseline. With this device, it is a very simple matter to accurately and rapidly set the meniscus exactly on the calibration mark without any secondary adjustment whatever. Moreover, the use of a mouthpiece is entirely eliminated.

In microanalysis, the Pregl[1] type of pipette is habitually used. Experience with this type of pipette has shown that two difficulties are encountered. The first is the disadvantage encountered with blood-dilution pipettes, viz., that the meniscus is difficult to adjust accurately since the zero mark is placed on a greatly constricted portion of the tube. The second lies in the fact that if, as is usually done, the pipette is sucked full to a point above the mark, and allowed to drop to the mark, the tube is wet above the mark, and on the subsequent washing out, this liquid is also washed out. While this is a small error, it prevents the highest precision from being attained.

At first we intended to make a series of pipettes equipped with syringes as an integral portion of the apparatus, as was done with the blood-dilution pipette. Since our needs were for a graded series of pipettes having a range of volumes from about 0.01 c.c. to 0.1 c.c. and a considerable number were desired, it would have been very laborious to construct all these as planned. We have instead made the pipette series as illustrated in Fig. 1, *B*, from capillary tubing, and in the top of each we have fitted a needle from a tuberculin syringe of 0.5 c.c. capacity, and sealed it in place with cement. The tuberculin syringe itself can now be used with an indefinite number of pipettes, and the difficulties above enumerated are entirely overcome. Pipettes are easily constructed in such a manner as to have any desired volume and almost any desired accuracy. The

*From the Division of Biochemistry, University of California Medical School.
Received for publication, February 2, 1932.

capillary is somewhat constricted at the point where the calibration mark is placed, so that the unavoidable error of reading may be reduced to almost any desired value. Too great constriction gives rise to difficulties due to capillarity. If small enough capillary tubing is available, this constriction is unnecessary. An error of 0.1 mm. in reading the meniscus causes an error of 0.02 c.mm. if the bore is 0.5 mm. in diameter. The pipette must be calibrated with mercury, and washed out in use as is done with the Pregl type of pipette. It must be mentioned that the same syringe and general construction are equally applicable to much larger pipettes than those built by us.



A Fig. 1. B

A similar method of manipulating pipettes has been suggested by Duff.[2] These were of the ordinary macro variety, where fewer advantages of speed and accuracy are gained than with those described by us. Moreover, his arrangement was adapted to use with bacterial cultures, largely for the sake of safety rather than to achieve extraordinarily fine manipulative technic. The rubber connections used in that design would make it next to impossible to calibrate a pipette with mercury, as we have found from experience, since the great suction exerted by a column of mercury distorts the rubber connection, and makes an accurate adjustment of the meniscus very difficult. Both the uses and design of

our apparatus are different, and we believe the advantages to be gained here are even greater than those gained by Duff in the solution of his particular technical difficulties.

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1. Pregl, F.: Quantitative Organic Microanalysis, English ed. 2, Philadelphia.
2. Duff, D. C. B.: Improved Pipette Manipulator, J. Lab. & Clin. Med. **15**: 1027, 1930.

NOTES ON APPARATUS FOR PERFORMING THE KLINE TEST*

Thomas B. Magath, M.D., Rochester, Minn.

THE increasing popularity of the Kline test and its use in large institutions has made it desirable to develop certain apparatus to facilitate the performance of the test.

PIPETTES FOR ANTIGEN

The pipette for the antigen, as recommended by Kline, is made by pulling out a piece of glass tubing so that the tip has an aperture which will permit a drop of antigen of from 0.0075 to 0.0085 c.c. to be delivered. This pipette is very delicate and easily broken. A permanent pipette (Fig. 1) can be made by drawing down a piece of 5 mm. glass tubing and sealing into the attenuated end

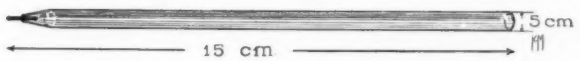

Fig. 1.—Pipette for antigen, with shaft of gold needle sealed into tip.

a half inch length of a gold needle shaft of number 21 gauge. The end of the needle after being cut off squarely is polished down until the wall becomes thin enough to allow a drop of antigen of 0.008 c.c. to be delivered; the size of the drop is determined not so much by the diameter of the opening as by the thinness of the wall at the tip. Such a pipette will deliver the exact amount of antigen indefinitely.

HOLDER FOR SLIDES

Fig. 2 illustrates a convenient and simple slide holder so that the slides after receiving the serum and antigen can be readily picked up from the table without danger of spilling. The holder is made of any suitable thin metal and is folded as indicated in the figure. The slide should project a half inch beyond the margin of the metal.

HEATER FOR PARAFFIN

Rings of paraffin, requiring the use of melted paraffin, must be made on glass slides in order to perform the test. To give the best results, paraffin should be at a temperature of about 165° C. Since this approaches the temperature at which paraffin catches fire, and since paraffin of this temperature will blister the skin rather badly, an electric heater has been designed (Figs. 3 and 4), so

*From the Section on Clinical Pathology, The Mayo Clinic, Rochester, Minn.
Submitted for publication, February 3, 1932.

that the heated paraffin will be kept at a relatively constant temperature with no danger of being set on fire and no danger of spilling. To make the box, sheet iron of number 16 gauge is folded as illustrated, and the ends welded in. It is insulated on the inside with asbestos, and on this is placed a simply wound heating element of nichrome wire. Suitable steel encased heaters can be purchased on the market with a wattage of 150. Near the heating element is placed a simple biometallic thermostat such as is used in heating pads. On the top of this is now placed another sheet metal box with welded ends. Asbestos is packed in around this and a sheet of asbestos is placed on top between the two metal boxes. Once the thermostat is properly adjusted no further care is necessary.

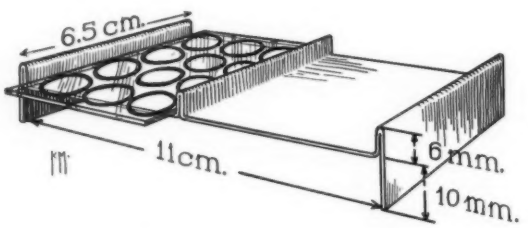

Fig. 2.—Frame to hold slides.

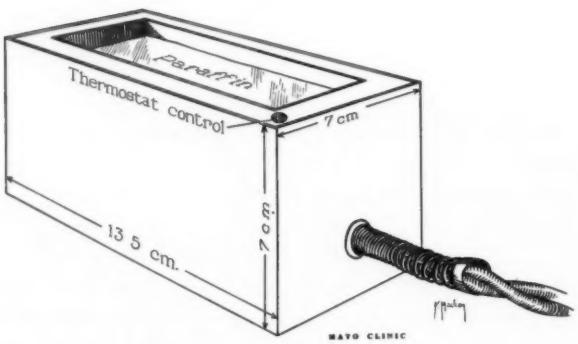

Fig. 3.—Heater for paraffin.

MACHINE* FOR TILTING AND SHAKING SLIDES

After the antigen and serum are mixed, the slides must be shaken, and the edges tilted in rotation without revolution for three minutes, so that all edges of the slide are tilted in rotation every second. The motion imparted to the slide will be clearly described later. When large numbers of these slides have to be used rotation by hand becomes very laborious and tiresome; in addition to that it is impossible to keep up a steady and constant speed of tilting in rotation. Therefore, to perform this act, a machine has been designed which may be understood by reference to Figs. 5 and 6 and the following description:

A-A designates two of the four pan-shaped containers or slide holders mounted on the plate *B*, which is secured in the inner ring of the ball bearing *C* the outer ring of which is in turn secured in the plate *D*. Plate *D* is mounted on and secured to the semicircular piece *G* mounted in the housing *J*. The piece

*This machine was designed and built in The Mayo Clinic machine shop by Mr. George G. Little.

G is graduated at *G* for the degree of tilt to which the plate holders *A-A* may be set, accomplished by and through the action of the worm thread on the shaft *H* meshing in the circular rack of the piece *G*. It is turned by the knurled head *I*. An angle of 15° is correct for performing Kline tests. The stationary shaft *F* is mounted securely in the base of the machine *Q* and passes up through the

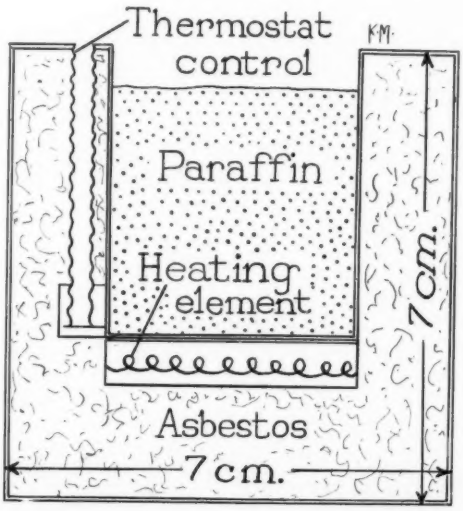

Fig. 4.—Heater for paraffin; cross section.

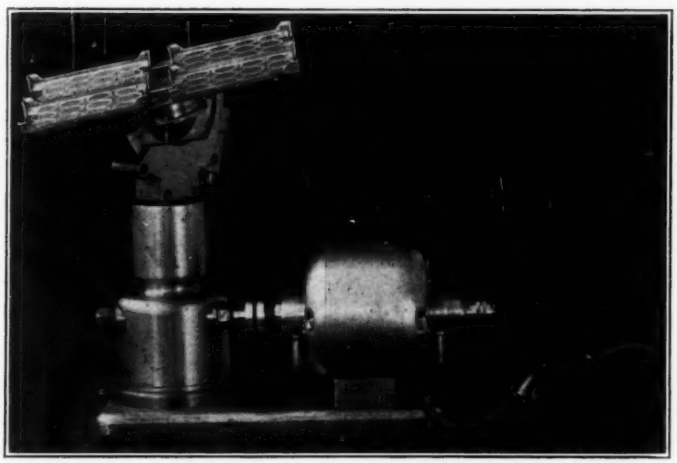

Fig. 5.—Machine for tilting and shaking slides.

hollow spindle or shaft *K* to the universal joint *E*. The universal joint *E* is supported by four pivot screws, two being in the yoke at the upper end of the shaft *F* and two in the hub or trunnion of the plate *B* within the inner ring of the ball bearing *C*. The housing *J* is secured to the main driving shaft *K*, rests thereon, and is caused to revolve when the shaft *K* is set in motion. Shaft *K* is located in suitable vertical bearings *T* upper, and *U* lower, mounted in the main housing of the machine and supplied with proper, long-time lubrication oil

cavities. Shaft *K* is supported endwise with a ball-thrust bearing that sustains the weight and provides antifriction in conjunction with the upper bearing *C*, thus making it possible to use a very small, low-powered motor to drive the machine. Shaft *K* has a worm wheel (not shown) mounted to mesh with and be driven by the worm *N* on the small shaft in the bearings *V* and *W*, the worm *N* is also provided with a ball-thrust bearing *M*. The part *P* is a detail of construction in which is located the lower vertical shaft bearing and facilitates assembly of the machine. The part *R* is a small flexible coupling connecting the worm shaft *N* to the motor *S*, which is a cheap fan motor. In action the glass plates being placed in the holders *A-A*, the worm *H* is turned to bring the holders into the proper or desired angle as indicated at *G*, the motor started to

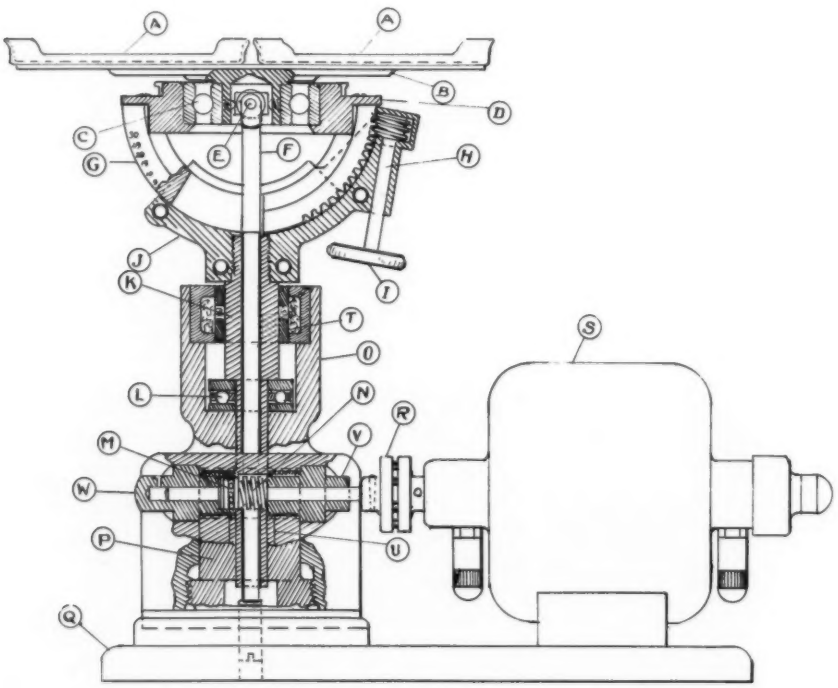

Fig. 6.—Machine, in section, for tilting and shaking slides.

cause the shaft *K* to revolve, carrying the housing *J* with it and also causing the outer ring of the ball bearing *C* to revolve, which, due to the fact that the level of zero shaft of *G* and the ring *C*, plate *B* and holders *A-A* have been changed to an angle with the shaft *K* and shaft *F* imparts an oscillating motion to the plate *B*. The inner ring causes the universal joint to assume a like angle, permitting the shaft *F* to remain stationary although the parts *K*, *J*, *G*, *C*, *B*, and *A-A* are caused to revolve. It is the action of the universal joint and the stationary position of the shaft *F* holding the plate *B* and the inner ring of *C* stationary that permits the outer ring of *C*, together with *G* and *K*, to revolve and impart a continuous tilting motion to *A-A*.

This movement is very simple and is easily understood if one thinks of the end of a round shaft cut off at an angle other than a right angle; then, while

it is revolved in a vertical position, if a rectangular plate is held against the end surface of the shaft, at the same time preventing the plate revolving, the motion imparted to the plate will be like that of the machine herein described.

The machine may be used to perform all manner of slide agglutination tests and will give great constancy in performing the Kline tests, both on serums and on spinal fluids.

THE VALUE OF SITOSTEROL AS A FORTIFYING AGENT FOR BEEF HEART ANTIGEN USED IN THE PRECIPITATION TEST FOR SYPHILIS*

B. S. LEVINE, PH.D., CHICAGO, ILL.

IT IS now known that cholesterol is not the only substance which increases the antigenic strength of alcoholic beef heart extract, but that this property is possessed by several of the sterols. Eagle[1] has made a study of this subject. He selected 50 water-insoluble alcohol-soluble substances and tested them for their antigen fortifying properties. Nineteen of the substances had to be discarded for technical reasons. Of the remaining 31, 16 proved to possess slight, 7 fair, and 8 very good fortifying qualities. First on the list of the 8 stood cholesterol, a reagent well known to serologists. It was followed by sitosterol. The latter belongs to a group of phytosterols, and as used by Eagle constitutes the water-insoluble alcohol-soluble nonsaponifiable fraction of wheat germ. In conjunction with cholesterol this reagent proved to possess ample sensitization power in Eagle's experiments.

The final reagent used in the experiments referred to consisted of the concentrated beef heart extract to which were added 0.8 per cent cholesterol and 0.8 per cent sitosterol. This supersaturation is accomplished by boiling. Upon cooling, the excess of cholesterol and sitosterol crystallizes out. It is, therefore, necessary to heat the reagent at 56° for some minutes before the antigen-saline suspension can be prepared for use as per titer. Eagle concludes that "the Wassermann reaction carried out with this antigen appears to be the most sensitive of biologic tests," since a 1:10,000 dilution of the antigen sufficed to give a four-plus fixation with a strongly positive serum specimen.

It must be stated at this point that such high degree of fortifying power is not limited to sitosterol, nor is it a result of supersaturation of the beef heart extract with the sterols. On several occasions I have prepared a highly lecitholized Kolmer antigen, fortified by the addition of only 0.2 per cent cholesterol, the primary titer of which was 1:10,000. The Kolmer antigen used in the laboratory of the Institute at this time is 0.5 c.c. of 1:1,000 dilution, and this, of course, is ten times the primary titer. In view of the above, the statement that "there is reason to believe that this antigen (cholesterol-sitosterolized) possesses almost the maximum sensitivity obtainable" and that "any further improvement must await the discovery of better sensitizers," appears unwarranted.

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*From the Clinical Laboratory, Public Health Institute.
Received for publication, February 29, 1932.

The experiments referred to apparently were designed to show that excessive sensitization by a fortifying agent is not a potent danger, and that with the combination of cholesterol and sitosterol, it is possible to continue to increase the efficacy of the antigenic reagent, with no danger of giving falsely positive tests. Such a view must necessarily rest upon the assumption that the complement fixation and antigen precipitation reactions, based upon fortified beef heart extract, are immunologic reactions specific for syphilis. There is still no unanimity of opinion on this point. My practical experience and experimental evidence lead me to the conclusion that the reactions are truly immunologic, but that they are specific for syphilis only when kept within certain zonal limits of reaction.[2] And the degree of fortification of the beef heart extract is one of the factors which determines the safe limits.

Antigen precipitation in syphilis follows, in a general way, the type-curve of colloidal precipitation. According to this type-curve, aggregation of the suspended antigen increases to a maximum with the increase in the content of any one of the constituents of the immuno-colloidal system of precipitation. Further increase in the content of the particular constituent leads to a progressive reduction in the aggregation of the antigen particles. The continued increase in the fortifying sterol content of the beef heart extract affects the precipitation reaction similarly. Therefore, a point is reached beyond which further addition of the fortifying agent to serums of weaker reaction (keeping the lipid content of the alcoholic extract constant) tends to retain the sensitized particles in a state of dispersion. The force that keeps the particles in dispersion is sufficiently strong to effectively resist the settling power of ordinary centrifugalization. Under such conditions, suspended antigenic particles sensitized by immunobodies other than of syphilitic origin, being generally of a low concentration, will remain in an invisible state of suspension. The same is true of suspended antigenic particles sensitized by syphilitic immunobodies of low concentration. It is evident, therefore, that in antigen precipitation, in which visibility is the sole criterion for the judgment of the positivity of a serum specimen, the danger of obtaining falsely positive results with an excessively fortified antigen is not very great.

The danger of bringing about nonspecific reactions in complement fixation is more potent than in antigen precipitation. The dilutions of the constituents used in this reaction, and of the antigenic reagent in particular, are high. The crest of the curve of sensitization and with it of complement consumption is not reached as readily. Continued increase in the sterol content of the beef heart extract, even beyond the point of normal saturation, continues to widen the zone of reactivity of the antigen. This is accomplished mainly through an increase in the surface area that has become activated to complement consumption. A point is finally reached at which the fixation of complement by antigenic particles sensitized by immunobodies other than of syphilitic origin exceeds one unit, making nonspecific fixation evident.

Abundant experience has shown that sensitization of the beef heart reagent and the proportionate fixation of complement may be of a truly immunologic nature, but may be caused by immunobodies other than those of syphilitic origin. The work of Kolmer indicates this.[3] It is also recognized by the

insistent demand for a "standardized technic." Experimentally this can be demonstrated by a laborious evaluation of the results of a large number of cases.[4] A quantitative comparison through a simple technic limited to one serum will prove only the increase in the intensity of the antigenic reaction of the beef heart extract with the increase in the content of the sterols. It will throw no light on the specificity of the highly fortified reagent. To arrive at the proper conclusion with regard to the limits of specificity in fortified beef heart extract, it is imperative that series of numerous complement-fixation tests be performed in which use is made of the same beef heart base fortified with cholesterol or other sterols from 0.1 per cent up to supersaturation (about 2.0 per cent at 56°).

Experimental evidence proving this point will be presented in a forthcoming report. The work reported in this paper is limited to the study of the value of sitosterol and of supersaturation as means for the fortification of antigen used in the precipitation reaction.

As a result of his studies on sitosterol as an antigenic fortifier, Eagle developed what he terms a more sensitive flocculation test.[5] The test briefly is as follows: An alcoholic beef heart antigen is prepared practically in the usual manner and is fortified by a combination of cholesterol and sitosterol to the point of oversaturation. One cubic centimeter of the fortified antigen is mixed with 1.3 c.c. of 4.0 per cent NaCl solution. This antigen-saline mixture is left at rest for thirty minutes to undergo a process which Eagle calls "ripening." Four hundredths of a cubic centimeter of this antigen-saline mixture is then placed into serologic tubes to which 0.4 c.c. of the inactivated serum is added. The tubes are properly marked for identification, and the set is shaken in the Kahn shaker for two minutes. Following this, the sets are incubated at 37.5° for four hours or longer. The tubes are then centrifugalized for ten minutes at a rate of 3000 r. p. m. One and two-tenths cubic centimeter of saline is then added to each tube, and the results read. A comparative study was made of the results obtained by this new precipitation test with several others. It was found that the new test possessed some technical advantages over the others, but that it did not attain the degree of sensitivity claimed for it.[6] Some serums from known syphilitic cases which were positive by the Kolmer and by the highly cholesterolized complement fixation and by the Kahn had to be recorded as negative and doubtful by the new test.

Close observation soon convinced me that sitosterol and cholesterol added jointly to the beef heart extract did not increase the sensitivity of the antigen as expected and that supersaturation with cholesterol alone was as efficacious as with a combination of the two fortifiers. It appeared, in fact, that saturation of the beef heart extract with either or both of the fortifiers is no requisite for a highly sensitive antigenic reagent; and, further, that supersaturation represents a point beyond the optimum for precipitation.

The following experiments were performed to verify the results of the preliminary observations.

Sixty-eight cases with histories of syphilis were subjected to the Eagle test using 0.04 c.c. of the antigen-saline suspension and 0.4 c.c. of the inactivated serum. The tubes were shaken for two minutes and incubated at 37° for four

hours. They were then centrifugalized, saline added, and the results read and recorded as described in another paper.[7] The same serums were subjected to the following procedure: 0.05 c.c. of the Kahn antigen-saline suspension was placed into Kahn tubes. To each was added 0.15 c.c. of the corresponding serum. The set was shaken for two minutes, placed in the incubator for four hours; the tubes were centrifugalized the same as for the Eagle tests and 1 c.c. of saline was added. The results are shown in Tables I and II.

TABLE I

NO.	EAGLE	KAHN 1ST	NO.	EAGLE	KAHN 1ST
1	2	0	35	4	2
2	4	2	36	3	4
3	4	4	37	4	4
4	2	0	38	4	4
5	4	3	39	2	2
6	2	0	40	1	1
7	2	P-M	41	1	3
8	4	P-M	42	3	2
9	4	3	43	1	1
10	3	1	44	P-M	3
11	4	4	45	4	4
12	4	4	46	2	0
13	4	3	47	2	1
14	3	1	48	4	2
15	4	4	49	4	2
16	4	0	50	4	4
17	3	0	51	4	4
18	2	1	52	3	2
19	2	2	53	3	2
20	2	2	54	3	3
21	4	4	55	3	4
22	4	4	56	4	4
23	2	4	57	4	4
24	2	1	58	4	4
25	4	3	59	4	4
26	4	3	60	4	4
27	4	3	61	1	2
28	2	2	62	4	4
29	0	1	63	0	2
30	2	2	64	4	3
31	P-M*	2	65	1	0
32	4	4	66	2	0
33	0	2	67	3	2
34	4	4	68	2	1

*P-M = Plus-Minus

TABLE II
RECAPITULATION

Total No. of tests	68
Total positives by the Eagle technic	64, or 94. % of total
Total positives by Kahn 1st, centrif.	58, or 85. % of total
Plus-minus by Eagle technic	2, or 3.0% of total
Plus-minus by Kahn 1st, centrif.	2, or 3.0% of total
Negative by the Eagle technic	2, or 3.0% of total
Negative by Kahn 1st, centrif.	8, or 12. % of total
Total average value, by Eagle technic	2.9
Total average value, by Kahn 1st, centrif.	2.4
Average of positives, by Eagle technic	3.1
Average of positives, by Kahn 1st, centrif.	2.8

It is seen from Tables I and II that the Eagle technic resulted in the greater number of positives (94 per cent) as compared with the Kahn (85 per cent). The general intensity of the reaction was also greater in the Eagle tests than in the centrifugalized Kahn first tubes (2.9 and 3 as compared with 2.4 and 2.8). However, the ratio of antigen to serum in the Eagle technic is 1:10, while in the Kahn first tube it is 1:3. The effect which the ratio of antigen to serum has upon the efficacy of antigen precipitation has been described by Porges et al.[8, 9] They showed that the aggregation of the antigenic reagent follows a type-curve as the ratio of the serum to antigen is progressively increased. Kahn showed that his 1:3 ratio, with very few exceptions, belongs to the midpart of the ascending portion of the curve, while the ratio of 1:10, also with some exceptions, is about at the crest of the curve.[10] It is not surprising, therefore, that the Eagle series, with an antigen to serum ratio of 1:10 gave more numerous and more intense precipitation results than the Kahn first tube, which represents a ratio of 1:3.

It is argued in one of the preceding paragraphs that supersaturating the beef heart extract with the fortifying sterols, according to the colloidal type-curve of precipitation, should lessen their original fortifying power in the precipitation test. That is, normally fortified antigen should prove more efficacious than supersaturated antigen in a long series of comparative tests, in which the ratio of the reagent to serum is the same for both antigens. To establish this, the following experiment was carried out: beef heart powder was extracted to complete freedom from ether soluble substances. It was then extracted with

TABLE III

NO.	EAGLE	LEVINE	NO.	EAGLE	LEVINE
1	4	4	31	P-M	4
2	4	4	32	1	4
3	0	1	33	4	4
4	4	4	34	2	4
5	4	4	35	4	4
6	0	3	36	2	4
7	4	4	37	4	4
8	4	4	38	0	2
9	4	4	39	4	4
10	0	2	40	2	3
11	4	4	41	4	4
12	0	4	42	3	3
13	4	4	43	4	4
14	4	4	44	0	1
15	2	2	45	0	4
16	P-M	4	46	0	2
17	4	1	47	2	2
18	4	4	48	P-M	1
19	4	4	49	P-M	3
20	2	2	50	4	4
21	P-M	4	51	P-M	1
22	0	4	52	4	4
23	4	4	53	0	4
24	4	4	54	4	4
25	4	2	55	1	2
26	4	3	56	4	4
27	3	4	57	4	4
28	4	4	58	2	3
29	4	4	59	4	4
30	1	4	60	4	4

alcohol exactly as for the preparation of the Eagle basic substance. This was fortified with 0.6 per cent cholesterol. The final reagent was not standardized, but was used in the test exactly as prescribed for the Eagle reagent and was used as per Eagle technic. This series is designated in Tables III and IV as the Levine series. Sixty tests were carried out on the same serums by the two technics. The results are reported in Tables III and IV.

TABLE IV
RECAPITULATION

Total number of tests	60
Positive by the Eagle technic	45, or 75.0%
Positive by the Levine technic	60, or 100.0%
Plus-minus by the Eagle technic	6, or 10. %
Plus-minus by the Levine technic	None
Negative by the Eagle technic	9, or 15.0%
Negative by Levine technic	None
Average Eagle value on the basis of the total	2.5
Average Levine value on the basis of the total	2.9
Average Eagle value on the basis of positives	3.4
Average Levine value on the basis of positives	3.8

From the comparison of the results of the two sets of tables it is seen that when the ratio of antigen to serum in the two procedures is the same, the antigen containing 0.6 per cent cholesterol yields precipitation results which exceed the results obtained by the Eagle antigen (supersaturated with cholesterol and sitosterol) in number (100 per cent as compared with 75.0 per cent) and in intensity of reaction (2.5 and 3.4 as compared with 2.9 and 3.8). It is evident from this that the use of sitosterol to obtain high sensitivity in precipitation tests is unnecessary. The fact that sitosterol and many other substances possess antigen fortifying qualities becomes of theoretical interest and importance. Since cholesterol is more efficacious as an antigenic fortifier than is sitosterol, as shown by Eagle and by the experiments here reported, and since the object in any laboratory technic is simplification, it is best that cholesterol alone continue to be used as the fortifying agent in the antigen precipitation tests for syphilis.

The results recorded in Tables III and IV indicated that by varying the antigen to serum ratio a suitable diagnostic reaction-level can be attained with the lowest percentage of sterol compatible with unmistakable visibility of centrifugalized positive reactions. Numerous series of experiments were carried out. The following procedure was found the most appropriate for practical laboratory purposes: Beef heart powder was extracted to complete freedom from ether-soluble substances. It was then extracted with alcohol for several days at room temperature. The filtered alcoholic extract was fortified with 0.4 per cent cholesterol and standardized so as to be used in a 1:1 ratio with saline. In the test proper 0.0125 c.c. of the antigen-saline mixture and 0.1 c.c. of the serum are used. No shaking by machine and no incubation at 37° are resorted to. After centrifugalization, 1 c.c. of saline is added to each tube and the results

observed. This procedure forms the basis of a centrifugoprecipitation test now in use in the laboratory of this Institute in conjunction with other accepted tests. The details of the newly developed precipitation procedure will be reported elsewhere.

In Table V a series of results obtained by this procedure are compared with those of the Kahn two-tube precipitation[11] and the Kolmer and cholesterolized complement-fixation.

TABLE V

NO.	LEVINE	KAHN	KOLMER	CHOLESTEROLIZED
1	4	4.4	4.4	4.4
2	4	Tr.2	4.4	2.0
3	4	4.4	4.4	4.4
4	4	3.3	4.2	1.0
5	4	4.4	4.4	4.4
6	3	Tr.Tr.	4.4	4.4
7	3	3.3	4.4	4.4
8	3	Tr.Tr.	4.4	4.3
9	4	4.4	4.4	4.0
10	4	4.4	2.2	0.0
11	3	0.1	4.4	4.0
12	4	4.4	4.4	4.4
13	4	4.4	4.3	3.0
14	4	4.4	4.4	4.4
15	4	0.0	4.1	0.0
16	2	0.Tr.	4.1	0.0
17	4	4.4	4.4	4.4
18	4	4.4	4.4	4.4
19	2	0.0	3.1	0.0
20	3	4.4	4.4	4.4
21	4	4.4	4.4	4.4
22	3	Tr.3	4.4	4.4
23	4	4.4	4.4	4.4
24	4	Tr.3	2.2	0.0
25	1	0.0	0.0	0.0
26	P-M	0.0	0.0	0.0
27	4	4.4	4.4	4.4

An equal number of known negative serum specimens were run simultaneously. The positive results in this procedure, no matter what their intensity were distinguished from the negatives with remarkable facility. So easy was the visibility that the results of the tests could be noted and called off faster than the recording person was able to write them down. A consideration of the results of the experiment recorded in Table V together with a study of the histories of the cases from which the serums were taken prove that fortification of the antigenic principle with a cholesterol content as low as 0.4 per cent (as compared with Eagle's 0.8 per cent cholesterol and 0.6 per cent sitosterol) suffices for the detection of syphilis antibody by the centrifugoprecipitation method. It substantiates the conclusions arrived at from the preceding experiments that the use of sitosterol adds nothing of intrinsic value to antigen precipitation, and that saturation and supersaturation of the alcoholic beef heart extract with the fortifying sterols is no requisite for ample sensitivity.

CONCLUSIONS

1. Supersaturation of the beef heart extract with sterol fortifiers does not bring out its maximum efficacy in the precipitation test for syphilis.

2. A redispersion of the sensitized particles results from, and a protective influence is exerted by the excess of fortifier which is sufficiently strong to prevent the settling of the sensitized complex through ordinary centrifugalization.

3. The addition of sitosterol to cholesterolized antigen is of no intrinsic value in the antigen precipitation test.

4. The principle of simplicity in laboratory technic would, therefore, seem to demand that cholesterol alone continue to be used as the antigen fortifying agent in the tube-precipitation test for syphilis.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

BLOOD: The Neutropenic State, Doan, C. A. J. A. M. A. **99**: 194, 1932.

The author presents a comprehensive yet succinct review of conditions associated with decrease in the number of leucocytes, especially agranulocytosis, presents illustrative cases, and the following conclusions:

The neutropenic state may be chronic or acute, constant, recurrent. In diagnosis it must be sharply differentiated from the leucopenia associated with many other clinical syndromes.

The chronic condition of moderate leucopenia may either mean a normal physiologic equilibrium maintained at a level somewhat lower than the average, with no detectable influence on the normal health of the individual, or it may reflect a low myelocytic reserve in the marrow with constant potential danger of marrow insufficiency and decompensation.

The acute or malignant neutropenic state presents a crisis that is rapidly fatal if recovery of marrow function is not initiated promptly. The underlying pathologic condition may include myeloid hypoplasia or hyperplasia.

Experimental and clinical observations form the basis for attributing to nucleic acid and to the nucleotides chemotactic, maturative and initiatory stimuli for neutrophilic myelocytes when the basic mesenchymal tissues from which they arise are in a condition to respond.

Blood transfusion and irradiation may act through the same nucleotide mechanism, when effective. The latter, because of its potentially destructive affinity for hematopoietic tissues, must be used with great caution when stimulation is desired. Further experimental studies are needed to ascertain whether the x-rays may be really primarily stimulatory to myeloid tissue without a preceding destructive phase.

BLOOD: Clinical Significance of Volume and Hemoglobin Content of the Red Blood Cell, Haden, R. L. Arch. Int. Med. **49**: 1032, 1932.

The following summary is presented:

1. The accurate determination of the volume, hemoglobin content and hemoglobin concentrations of the erythrocyte is a relatively simple procedure and should be carried out in every case of anemia that requires careful study.

2. The results are best expressed as indexes rather than in absolute figures. The indexes express the absolute values relative to normal.

3. In normal men and women the indexes are always within the limits of normal (0.09 and 1.10).

4. Every case of anemia that warrants thorough study should be classified on the basis of the number, the volume and the hemoglobin content of the red blood cells.

5. A simple classification and terminology on the foregoing basis is suggested.

6. The typical determinations in different clinical groups of anemia are reported.

7. In the case of acute mechanical blood loss there is a change in the number of cells only; in chronic loss the cells become progressively smaller, with a low hemoglobin content.

8. If the anemia is due to accelerated destruction, the mean cell volume tends to increase since the reticulocytes are usually larger than normal.

9. With decreased blood formation the determinations are very variable. The mean cell volume and hemoglobin content are seldom greater than normal except in pernicious anemia.

10. In pernicious anemia an increase in volume and hemoglobin content is the most characteristic observation on the blood.

11. The volume and hemoglobin content are important aids in prognosis.

12. In pernicious anemia the mean volume of the erythrocyte is the best index in treatment. If the patient is adequately treated, the mean cell volume returns to normal.

13. In the treatment for other types of anemia the volume and hemoglobin content of the red cell are valuable aids in treatment.

RHEUMATIC FEVER: Streptococci in the Blood in, Lichtman, S. S., and Gross, L. Arch. Int. Med. **49**: 1078, 1932.

Study of 5,233 consecutive blood cultures in a general hospital shows that with adequately sensitive methods an incidence of nonhemolytic streptococcemia (alpha and gamma types) between 4 and 15.5 per cent with an average of 6 per cent occurs in at least 9 diseases, i. e. acute rheumatic fever with polyarthritis, chronic rheumatic cardiovalvular disease, rheumatoid arthritis, aplastic anemia, pernicious anemia, leucemia, colitis, meningococcus meningitis and pyelitis and pyelonephritis. On the basis of the incidence of the ''transient'' streptococcemia alone, these organisms cannot justifiably be considered as the causative agents of these diseases.

BIOPSIES: Suction in Obtaining Endometrial, Klingler, H. H., and Burch, J. C. J. A. M. A. **99**: 599, 1932.

The method is as follows:

An ordinary Keyes-Ultzman cannula which in reality is nothing more than a rigid hollow uterine sound with perforations at the tip, was used.

While the results with this instrument have proved satisfactory in a number of cases, it was frequently difficult to insert the instrument on account of its rigidity. A Luer or all-glass type of syringe was attached to the cannula. However, numerous syringes were broken in withdrawal and suction due to the stress at the junction of syringe and cannula. This made it necessary to substitute a tube constructed of malleable copper and attached to a record syringe.

The technic is briefly as follows:

The patient is placed in the lithotomy position. Cervical exposure is obtained by means of a bivalve speculum. The cervix is cleansed of mucus and the os painted with mercurochrome-220 soluble. The tube is inserted into the uterus and bent slightly, depending on its position. When the cannula comes to rest against the uterine wall, the syringe is attached to the cannula and strong suction is made, while slight pressure keeps the tip of the cannula in contact with the uterine mucosa. While suction is maintained, the cannula is withdrawn for about half an inch and then released. The instrument is then rotated slightly so that on reinsertion it will come to rest on another part of the mucosa. This process is usually repeated about three times, and the instrument withdrawn during continuous suction. The apparatus is carefully washed with solution of formaldehyde by drawing the fixative back and forth several times in order to remove all the particles of tissue from the apparatus. It is then carefully washed out and sterilized and is ready for use again.

CHOLESTEROL, Blood, in Hepatic and Biliary Diseases, Epstein, E. Z. Arch. Int. Med. **50**: 203, 1932.

Improvement in the accuracy of diagnosis and prognosis of various hepatic and biliary diseases is possible by means of a quantitative study of the blood cholesterol and cholesterol ester.

In obstructive jaundice, hypercholesteremia is usually encountered. It roughly parallels the degree of obstruction and the bilirubinemia and returns to normal with relief of the obstruction. Exceptions are noted in cases of marked cachexia, cholemia and superimposed infections. The cholesterol esters in mechanical obstruction rise concomitantly with the total cholesterol in about half the cases; in the other instances they remain normal but lag relatively behind the increased free cholesterol.

In degenerative diseases of the liver, a pronounced divergence between the bili-

rubinemia and cholesteremia usually occurs; the more severe the damage to the liver, the greater the tendency to hypocholesteremia. This divergence between the hyperbilirubinemia and the cholesteremia offers a means of differentiation from the cases of mechanical obstruction. In parenchymatous degeneration of the liver, a drop in cholesterol esters parallels the severity of the damage even more accurately. In the rapidly fatal cases the cholesterol esters are very low or absent throughout the course of the disease; in less severe cases, the initial low ester values eventually rise with improvement in the condition.

In atrophic cirrhosis of the liver (Laennec), the cholesterol blood pictures remain normal. Variations occur only when hepatitis or degeneration of the liver are superimposed in the terminal stage of the disease.

In cholecystitis and cholelithiasis with no obstruction to the biliary outflow, the blood cholesterol figures are normal or insignificantly elevated.

PLASMA PROTEIN, in Relation to Blood Hydration, Peters, J., Bruckman, F. S., Eisenman, A. J., Hald, P. J., and Wakeman, M. J. Clin. Invest. **11**: 97, 1932.

I. Proteins in Acute Nephritis:

Serum proteins have been determined 85 times in 38 cases of acute nephritis.

Edema was regularly found when the protein concentration was below 4 per cent; but was sometimes present in the early stages of the disease when protein and albumin were within or just below the normal limits.

In the early stages of the disease edema appears to be due partly to vascular disturbances which increase the hydrostatic pressure or the permeability of the capillaries.

Serum albumin reductions, when they occur, seem to be referable to leakage of albumin into the urine and malnutrition.

Serum globulin in acute nephritis is frequently above normal.

II. Serum Proteins in Heart Disease:

In patients with heart failure serum albumin is frequently reduced.

Although edema of heart failure may occur even when serum protein and serum albumin are at or above the normal level, it is more commonly associated with some degree of albumin deficiency.

The albumin deficits appear to be directly referable to malnutrition.

III. Serum Proteins in Terminal Stages of Renal Disease:

Reduction of serum proteins at the expense of the albumin fraction is common in the terminal stages of renal disease.

The serum albumin deficiency cannot be correlated with the incidence of edema, which is usually referable to heart failure.

Wasting is a characteristic feature of the condition and can be correlated with the serum protein deficiency, unless some other functional disturbance has produced hemoconcentration.

SERUM DISEASE, Recent Observations in, Hunt, L. W. J. A. M. A. **99**: 909, 1932.

Serum disease occurred in 28.1 per cent of 2,859 patients who received diphtheria antitoxin, in 22.7 per cent of 858 patients who received scarlet fever antitoxin, and in 81.8 per cent of 55 patients who received antimeningococcus serum.

The occurrence of a serum reaction after the injection of diphtheria and scarlet fever antitoxin is determined in part by the susceptibility of the individual, by the toxic properties of the serum, and, in the largest measure, by the total quantity of serum given. Concentrated diphtheria antitoxin calls forth reactions in about the same proportion as does whole serum in corresponding bulk.

The serum reactions after the use of scarlet fever antitoxin were slightly less in frequency than those after the use of diphtheria antitoxin. They were not more severe.

The incidence of serum disease does not vary widely in the various age groups.

The interval between the injection and the appearance of the reaction varies from a few minutes to thirty days. The majority of the reactions appear before the eleventh day.

TRANSFUSION, Blood, Results of 1500 Transfusions in 1000 Cases, Polayes, S. H., and Morrison, M. Am. J. M. Sc. **184**: 326, 1932.

Of 1000 cases receiving a total of 1500 blood transfusions the results were either of doubtful benefit or of no effect whatever in 71.7 per cent.

Blood transfusion proved to be a life-saving procedure in 1.4 per cent of the cases. Most of these were patients who suffered a sudden loss of a large quantity of blood.

Some definite adjuvant beneficial effects were derived from transfusion in 23.3 per cent of the cases.

The reaction to the transfusion was unfavorable and the results harmful in 3.7 per cent of the cases.

The transfusion was apparently contributory to death in 0.9 per cent of the cases.

The excellent results from blood transfusion in the wide variety of diseases as reported by other writers could not be obtained in this investigation. Definitely beneficial results should be expected only in the type of case indicated in this study.

ARTHRITIS, Significance of Sedimentation Rate of Blood Corpuscles in Synovial Fluid and Plasma, Kling, D. H. Arch. Int. Med. **50**: 419, 1932.

The following technic was used:

Preparation of the Suspension of Blood Corpuscles in Synovial Fluid.—Into a graduated centrifuge tube is put 2 c.c. of 3.8 per cent sodium citrate solution, and then the amount is increased to 10 c.c. with synovial fluid. Into a second graduated centrifuge tube 3 c.c. of the sodium citrate solution is put, and venous blood is added to 15 c.c. The amount can be reduced in the ratio of one part of citrate to parts of synovial fluid or blood; the minimum required is 5 c.c. of citrated synovial fluid and 10 c.c. of blood, however. Six cubic centimeters of the citrated blood is then transferred to a separate test tube and centrifuged at a high speed, simultaneously with the citrated synovial fluid. After ten minutes of centrifugation, the tube containing blood is marked at the level of separation of the corpuscles from the serum. A second mark identifies the upper level of the plasma. In order to be assured that the separation of the cells and plasma is complete, recentrifugate until there is no change in the level of the cells. The synovial fluid is centrifugated until the supernatant layer is clear. The plasma is then separated carefully from the corpuscles with a pipette, and an equal amount of centrifugated synovial fluid is substituted, to the upper mark. This procedure secures a suspension of corpuscles in synovial fluid equal to that in the citrated blood.

The Set Up of the Sedimentation Test:

Two 5 c.c. pipettes are marked at a height of 20 cm. from the tip. Holes to a depth of about 5 mm. are bored in large rubber stoppers (No. 10).

The suspension of corpuscles in the synovial fluid and the remainder of the original citrated blood are now shaken until thoroughly mixed. Each is then drawn into a pipette to the mark and set up vertically in a rubber stopper; plasticine can be used for this purpose, if desired. At intervals of one hour, marks are made at the level of separation of the corpuscles from the clear fluid. Final readings are made in twenty-four hours.

This method follows the Westergren technic except for two modifications: (1) citrate is accurately measured into tubes instead of being drawn into syringes: (2) the pipettes are of larger caliber because of the higher viscosity of the synovial fluids. Finally, more readings were made than in the routine tests, in order to secure more data.

On the basis of a study of 61 cases of arthritis by this method, the following conclusions were drawn:

The severity of an infection of the joint is indicated by the sedimentation curve in the synovial fluid; the general reaction is reflected in the blood curve.

In acute infectious polyarthritis, the comparative sedimentation index is useful in the determination of the part played by the aspirated joint within the general process.

In monarticular arthritis, a high increase in the blood sedimentation and a low sedimentation rate in the synovial fluid indicate foci of infection outside of the joint as responsible for the increase in the sedimentation in the blood.

A simultaneous determination of the sedimentation and the viscosity is helpful in differentiating the type of involvement of the joint.

Noninflammatory fluids with a low protein content have a low comparative sedimentation index and a low viscosity. This is the case in transudates. On the other hand fluids with a high content of mucin show a low comparative sedimentation index but a high viscosity.

The differentiation of infectious from degenerative types of chronic arthritis by the sedimentation of the blood alone is not possible. The nature of the underlying process can, however, be more accurately discovered by the comparative sedimentation and the viscosity of the effusion.

Note: This method of comparative sedimentation was also used with other body fluids (pleural, pericardial and ascitic effusions). Four cubic centimeters of citrated blood is sufficient, as these fluids have a low viscosity. Two cubic centimeters is centrifugated, and the plasma is replaced by an equal volume of the citrated fluid to be examined. The tests are set up in the standard pipettes for the Westergren method. The findings will be reported elsewhere.

SCARLET FEVER, A Survey, Peacock, S. C., Werner, M., and Colwell, C. Am. J. Dis. Child. 44: 494, 1932.

Of 258 adults examined, hemolytic streptococci were isolated from 72, or 28 per cent, and 45 or 17 per cent, gave positive skin reactions.

Forty of the 45 who gave positive skin reactions received toxin for immunization. Of these retested, 87.5 per cent were immunized to the point of negative skin reactions.

On the basis of both time off duty and symptomatology, 15 per cent of the people immunized experienced severe reactions. Approximately 85 per cent of the subjects receiving injections of toxins had more or less distinct reactions.

Hemolytic streptococci had disappeared from the nose and throat of 92 per cent of the subjects surveyed at the end of twenty-eight days. Only 15 per cent of the susceptible subjects, as determined by the Dick test, harbored organisms.

Among the 258 subjects with positive or negative cultures, the number with tonsils and that in whom tonsillectomy had been performed were approximately equal.

The erythema of the skin test averaged 19 by 18.6 mm. in diameter; its size bore no particular relation to the grade of reaction experienced by the adults immunized.

Of the adults who gave negative skin reactions, 70 per cent gave no history of scarlet fever, 24 per cent gave a history of having had the disease, and 6 per cent furnished records with no data available on that question.

If the chocolate agar medium is a reliable means of differentiating nonscarlatinal from true scarlet fever streptococci, then 56.9 per cent of the organisms isolated in our series should be classed as related to scarlet fever.

If the opsonification test is of differential value 43 per cent of the organisms isolated were of scarlatinal origin, an agreement of 80 per cent with those giving no reaction on chocolate agar.

Among the 186 persons giving negative reactions to the Dick test, no definite case of scarlet fever developed.

Of the 40 adults giving positive skin reactions, 2 contracted scarlet fever before the series of immunization was completed, and none after the immunizations were complete. Of the 5 subjects who were not immunized, 2 had severe attacks of the disease.

RHEUMATIC FEVER, The Contagious Factor in the Etiology of, Collis, W. R. F. Am. J. Dis. Child. 44: 485, 1932.

The etiology of rheumatic fever can be explained only when its connection with acute sore throat caused by the hemolytic streptococcus is understood. It cannot be described as a contagious disease in the ordinary sense of the word, as more than one factor is responsible for its development. The streptococcal infection of the throat is no doubt contagious in the epidemic sense, but is followed by rheumatic fever only in certain patients. Although there is some evidence in support of the view that before rheumatic fever can develop, the patient must have been previously in a prerheumatic condition, characterized by a changed tissue reaction to the organism or its products, this hypothesis must for the present be considered sub judice.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Primary Carcinoma of Lung*

THIS is another of those authoritative and exceptionally useful publications which appear periodically as "Medicine Monographs."

The book consists of two portions, the first devoted to general consideration of primary carcinoma of the lung, and the second being devoted to a discussion of the subject from the clinical standpoint.

The first six chapters cover the incidence, etiology, histogenesis, classification, metastases, and duration of the disease.

From a thorough review of the literature and a rather extensive clinical experience Fried concludes that the more frequent occurrence of cancer of the lung in recent years is more apparent than real and associated with improved clinical and pathologic methods of diagnosis, the increased attention focused upon the condition, and the fact that, with the increase in the normal span of life, more people reach the "cancer age."

Bronchiogenic cancer is apparently merely a part of the problem of malignant disease and its true etiology as yet remains unknown.

Fried believes that primary carcinoma of the lung is bronchiogenic in origin and follows excessive regeneration of the bronchial tree after inflammatory processes; that it is primarily of basal cell origin, and results from protoplasm of the undifferentiated basal cell of the bronchial mucous membrane.

Classification of the tumors is difficult but may be regional, gross, or microscopic the latter being unsatisfactory and largely dependent upon the duration of the disease, which, apparently, runs a protracted course.

In the clinical portion of the book diagnosis, laboratory methods, and 47 illustrative cases are thoroughly discussed. The section should prove especially useful because, the disease being commonly regarded as rare, is not thought of and so undoubtedly often overlooked.

There are numerous excellent and well reproduced illustrations, all photographic.

The format is pleasing and the type easily read.

This book deserves an enthusiastic reception from the clinician as well as the pathologist and should lead to a better understanding of what is now known to be a far from uncommon disease.

Human Cancer†

THE subtitle of this book (Etiological Factors; Precancerous Lesions; Growth; Spread; Symptoms; Diagnosis; Prognosis; and Principles of Treatment), indicates its comprehensive scope.

As malignant tumors vary in many respects in accordance with the tissue from which they arise as well as the region of the body in which it is located, the arrangement of the book is by regions.

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*Primary Carcinoma of the Lung: Bronchiogenic Cancer: A Clinical and Pathological Study. By B. M. Fried, M.D. Cloth, 247 pages, 95 illustrations. Williams and Wilkins Co., Baltimore, Md.

†Human Cancer. By Arthur Purdy Stout, Associate Professor of Surgery, College of Physicians and Surgeons, Columbia University. Cloth, 1007 pages, 331 illustrations. Lea and Febiger, Philadelphia, Pa.

This, for purposes of reference, is both excellent and practical and will undoubtedly be received with favor by those to whom the book is addressed.

Each section is preceded by a discussion of the etiologic factors and precancerous lesions as at present understood. This is followed by a clear, succinct, and yet comprehensive discussion of the malignant lesions common to the tissue and region under discussion.

The book is profusely and excellently illustrated.

The recent and pertinent literature is well reviewed and commented upon in the light of an extensive experience. This is particularly notable in the discussions of diagnosis, and of the rapid biopsy methods and the criteria of the degree of malignancy as estimated by tumor cell morphology.

The author lays great stress upon the method of Laidlaw in which, by means of silver impregnation cells may be differentiated in accordance with their embryonic derivation.

This method will doubtless be widely used.

While primarily addressed to surgeons and surgical pathologists, the book is of great interest and appeal to all who come in contact with malignant lesions, and who does not?

The volume deserves a wide circulation as one of the most practical and useful tests at present available.

Quantitative Clinical Chemistry*

THIS volume without reserve may be accepted as the last work to date upon the subject of quantitative methods in Clinical Chemistry and, together with its companion volume, Quantitative Clinical Chemistry, Interpretations, for the first time furnishes a comprehensive and authoritative survey of a field in which many important advances have been made within the last decade or so.

In this book are presented methods for the determination of those substances; found in the body and its excreta, which are of importance for clinical medicine and for which suitable quantitative methods are available.

This, indeed, is an ambitious and comprehensive undertaking, but it has been carried out in a manner eminently satisfactory for those by whom this book will be used as a reference.

Appreciating that laboratory facilities, as well as technical aptitude, are subject to considerable variation in accordance with varying circumstances, the authors have presented in each instance methods of different types as well as, when advisable, both macro and micro methods. The book thus forms a practical manual of technic adapted to practically any and all situations.

The experience and reputation of the authors in this field is a sufficient guarantee of the reliability of the methods chosen by them for presentation, most of which they have, moreover, subjected to extensive practical trial under clinical conditions.

Preceding the technical description of each method is a discussion of the principles upon which the methods are based which is also in effect, not only a critical review of the methods described, but also a cross-section of the literature on the subject. The reader is thus informed as to additional methods available in the literature.

The first two chapters on General Chemical Technique and Special Biochemical Technique contain a wealth of practical experience and well repay the perusal of every worker in this field.

Indeed, this is true of all the thirty-two chapters and appendix which compose the book.

There is little doubt but that this volume will be the standard reference text for years to come.

The format and typography are excellent and the authors and publishers are to be congratulated upon the presentation of a volume for which the laboratory worker and the physician have long been waiting.

The book should be on the work shelf of every laboratory in which clinical quantitative chemical determinations are conducted.

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*Quantitative Clinical Chemistry, Volume II, Methods. By John P. Peters, M.D., Professor, Internal Medicine, Yale University, and Donald D. Van Slyke, Ph.D., Sc.D., Rockefeller Institute for Medical Research. Cloth, 957 pages, 94 figures. Williams & Wilkins, Baltimore, Md.

The Chemistry of Tuberculosis*

CONTRIBUTIONS to the study of tuberculosis in all its phases have been so varied, so voluminous, and so widely scattered in the literature that it is only by means of such comprehensive discussions and reviews as are embodied in the present volume that the student of this subject can hope to keep abreast of its many advances.

The present edition has been so completely and thoroughly revised as to constitute, to all intents and purposes, a new book.

The book is divided into three main sections. The first, devoted to the Chemistry of Acid-Fast Bacteria, presents in a comprehensive and authoritative manner all that is known at present of the metabolism of the tubercle bacillus and its relation to the phenomena of the disease.

In the second section, The Chemical Changes in the Tuberculous Host, is summarized, reviewed, and discussed, in both an orderly as well as an understandable manner the fruits of modern investigation of the manifestations of the disease correlated with the material discussed in the preceding portion of the book.

The final section is concerned with the Chemotherapy of Tuberculosis, discussed and evaluated on the basis of "biologic experimentation carefully coordinated with constructive chemical manipulation and chemical research of an advanced type."

The volume may be regarded as not only a distinct and valuable contribution to a most important subject but as indispensable for a thorough understanding of a complicated subject.

Clinical Endocrinology of the Female†

ONE of the most recent developments of modern medicine has been the gradual evolution of a beginning understanding of the many complexities involved in the interlocking functions of the group of structures comprising the so-called "organs of internal secretion."

So extensive have been the studies in this field within recent years and so great has been the value and importance of their practical application that in order to understand and apply them a new department of medicine, endocrinology, has come into being.

As is usual in the newer developments of medicine, much of the material from which present conceptions of endocrinology arise and upon which its practical applications are based, has appeared in more or less accessible and more or less specialized journals. The present volume by Drs. Mazer and Goldstein is eagerly welcomed as presenting under one cover a comprehensive and authoritative survey of endocrinology in its relation to the female, not only from the theoretical but also from the viewpoint of its practical application to the study

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*The Chemistry of Tuberculosis. By H. Gideon Wells, M.D., Professor of Pathology, University of Chicago, and Esmond R. Long, M.D., Professor of Pathology, University of Pennsylvania. Cloth, ed. 2, 481 pages, Baltimore, Md., Williams & Wilkins Co.

†Clinical Endocrinology of the Female. By Charles Mazer, M.D., F.A.C.S., Assistant Professor of Gynecology and Obstetrics, Graduate School of Medicine, Univ. of Penna. and Leopold Goldstein, M.D., Demonstrator of Obstetrics, Jefferson Medical College; 518 pages with 117 illustrations. Cloth. Philadelphia, and London, W. B. Saunders Co., 1932.

and management of such conditions as are directly or indirectly related to disturbances of the endocrine system.

Few conditions more commonly confront the practitioner at large than those related to disorders of the menstrual function, and the phenomena incidental to the menopause, while the number of those suffering from minor gynecologic disturbances is legion.

That in many such instances endocrinologic treatment has much to offer is generally known. But exactly *what* it offers and how or by what methods results may be achieved has been discoverable only by search of the literature.

This situation is changed by the appearance of this volume. Here the practitioner will find available all the proven and acceptable methods presented in a clear-cut and understandable manner.

Both the arrangement of the book and the manner in which the material is presented may be highly commended. Comprehensive without being verbose; succinct but not laconic; clear and without ambiguity, despite the complexities of the subject the book is easy to read and to understand.

The long experience of the authors and their connection with research in this field assures the reader that their presentation is authoritative.

Well illustrated and excellently printed, this volume may be recommended without reserve to the practitioner at large as well as to those whose interests are more specialized as an authoritative contribution to a complicated and very important subject.

The Journal of Laboratory and Clinical Medicine

VOL. XVIII ST. LOUIS, MO., OCTOBER, 1932 No. 1

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Richmond, Va.

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Entered at the Post Office at St. Louis, Mo., as Second-Class Matter. Additional entry as Second-Class Matter at Fulton, Mo.

EDITORIAL

What Price Doctors?

FIVE years ago a committee was organized by physicians and other interested persons to study and report upon the problem of the costs of medical care. The committee numbers around sixty, of whom about one-half are physicians, representing the practice of medicine, public health and industrial medicine, and the remainder are members of the dental and nursing professions, economists, sociologists, and representatives of insurance companies, charitable organizations and endowments and of the public at large. The committee has been headed by Ray Lyman Wilbur, himself a physician.

The devastating effect of illness upon the family pocketbook is obvious justification for such an investigation. This Committee, in close collaboration with The American Medical Association and several other organizations, is in the midst of a very detailed investigation of the entire subject. Booklets reporting their observations in the various fields are being published as the individual studies are completed, and a most valuable accumulation of facts is being made.

Very soon after the organization of the Committee, a familiar psychologic phenomenon made its appearance. The doctors had on their own initiative started this survey of their own organization to determine if possible what faults there might be and what might be the most reliable remedies. At once, non-medical reformers took up the cry. Each felt that he alone saw all the shortcomings of the organization of the medical services, and he alone could present the proper solution. The popular magazines were delighted to publish the articles which were usually based primarily on personal experience with illness in the writer's family or personal grievances, and which rarely showed evidence of any wide conception of the intricacies of the problem.

The doctors in general were content to ignore many of the invectives of these articles and to await the more intelligent and better informed reports of the Committee, which would be based on an accumulation and interpretation of facts rather than upon theory and fancy.

The objectives of the Committee have been (1) the accumulation of data on the needs for medical services, (2) data on the existing facilities, (3) study of the cost of adequate medical service, (4) investigation of the reason for the recently increasing costs of adequate care, (5) promulgation of a constructive program directed toward lowering the cost of medical care and (6) stimulation of interest in the problem on the part of the medical profession. Throughout the five years, reports have been made at frequent intervals covering various phases of the economics of sickness and medical practice, with no attempt being made to prematurely suggest radical changes or solutions. The work so far has essentially represented the taking of an inventory.

Most excellent work has been accomplished by the committee and a wealth of statistical data has been collected which can now be substituted as facts, for the previous guesswork of the theorists above mentioned. Whether this will bring us any nearer to a solution of the age-old problem of the economic distress associated with disabling illness is a question that only the future can answer. We look for no panacea and possibly no radical changes in present methods resultant on the investigation of the Committee, but they have already given us an abundance of facts upon which to cogitate and have pointed out some very definite situations obviously requiring correction.

The customary reply of the physician to the criticism that the costs of illness are too high, is that it is the other requisite services and materials and not the doctor's fee, that are responsible. Certain it is that in disabling illnesses requiring hospitalization and nursing care, the doctor's fee comprises a relatively small amount of the total cost.

Each of the agencies involved can however with equal justice make the same statement. Drug bills are often high, but if the druggist were to depend for his income entirely on the profits of his prescription service he would not prosper. The cost of a special nurse is ruinous to a meager pocketbook, but no nurse manages to do much more than make a decent living and far too many are unable to lay aside enough to keep them from want after their relatively early retirement. Hospital charges seem tremendous but in spite of this most of them require endowments and it is exceptional, even for the private institutions, to achieve what any business man would term an adequate return on his investment. Obviously,

therefore, all sources of expense to the patient may well be investigated, and it is possible that need for cutting expense may be found in any or all of these sources, or it may be found that the defect does not lie in any, but in the present day haphazard manner of providing for the possible future contingency of illness.

For the present discussion we shall leave the problem of other sources of expense to others, perhaps better qualified to discuss them, and shall confine ourselves rather to the question, are physicians' fees too high, is the physician receiving too large an income for his services?

R. G. Leland, Director of the Bureau of Medical Economics of The American Medical Association has made a survey of the incomes of physicians in the United States. It is based upon a representative series of 6,328 doctors, including those in general practice and those in the various specialities. Gross incomes ranged from as low as under $500 to as high as $160,499. The survey appears to represent a quite reliable cross section of the incomes of physicians in the United States. Of the 6,328, fifteen reported a gross income of under $500 and 165 of over $30,500. One reported a gross income of $160,499. The vast majority, nearly 6,000, reported gross incomes of between $500 and $30,500.

In medicine as in most other fields today the net income is considerably lower than the gross. The physician's overhead is not at all inconsiderable. Leland found that the average net income of the general practitioner is 67 per cent of his gross income. In the specialties where expensive equipment and larger offices are requisite, the net income falls proportionately.

The average gross income for all physicians, including the specialists and those who reported unusually large incomes, was $9,764.40. The physicians in general practice reported an average gross income of $7,781 with a net of $5,250.

The specialties of medicine showed distinct variations in gross incomes. The highest average was in surgery ($16,393) which was followed closely by roentgenology ($16,083). Following this, in order, came orthopedic surgery, dermatology, obstetrics and gynecology, toxicology, internal medicine, eye ear nose and throat, urology, pediatrics, pathology, insurance work, anesthesia, bacteriology, industrial medicine, general practice, neurology and psychiatry, tuberculosis, physiotherapy, teaching and public health.

Of the entire number of physicians approximately 20 per cent reported gross incomes of under $4,500. Fifty per cent showed gross incomes under $7,500, and 80 per cent had a gross income of under $13,500.

Let us compare present day doctors' incomes with those of ancient times. Galen, the leading Roman physician of his time, who lived in the second century after Christ, is reputed to have received a fee which would correspond in our present monetary standards to $6,000 for one consultation. The favorite physician of the Arab, Harun-al-Raschid, who reigned from 786 to 802 A. D. was paid $1,500 annually for "bleeding and purging the Commander of the Faithful." This was in addition to his monthly salary of $2,500 and a New Year's Purse of $6,500. His income therefore corresponded to an annual income today of $38,000. He, himself, estimated his entire fortune in fees as $10,000,000. He was banished for one reason or another but was recalled to heal El-Meismun, for which he received a fee of $125,000. During the reign of Charles VIII, Thierry

de Hery stated his income as 30,000 francs. Money at that time had about eight times the purchasing value of money today.

In the eighteenth century the incomes of doctors scattered over Europe appear to have ranged from $3,000 to $90,000 annually. Katherine of Russia paid the equivalent of $50,000 to have herself and her son vaccinated against smallpox, allowed an additional $10,000 for travelling expenses, and bestowed upon the physician a life annuity of $2,500.

Average incomes in bygone days may be guessed at from the following. In 1224 Frederick the Second in Sicily issued an edict requiring physicians to treat the poor for nothing. They were allowed to charge thirty-five cents a visit for office practice and for patients living inside the city. For calls made outside the city $3.00 could be charged if the physician paid his own expenses, while if the expenses were met by the patient, the fee was $2.25. It is said that the purchasing power of money at that time was from fifteen to twenty times that of the present. During the reign of Henry VII, Henry VIII, and Queen Elizabeth the Court Physicians received money valued at $200 annually. Money had about eight times its present purchasing value. In Germany at that time the consultation fee of physicians was $2.50.

The average cost of a good medical education runs in the neighborhood of $10,000. To this should be added the value of the money which the student would have been making during the years of his instruction were he not at school, and interest incurred in defraying the expenses of his education. With this the total cost becomes more nearly $20,000.

If we are to compare physicians' incomes with incomes from other businesses and professions we must discuss it in the terminology of capital and labor. First, reduction must be allowed for a fair interest on the original investment for education of from $10,000 to $20,000. Fair interest on an investment as business sees it today reaches more nearly 10 per cent than 6 per cent. From $1,000 to $2,000 should therefore be subtracted from the physician's net income before his income can be compared with that of the average man in the white collar class. He should also be allowed a 50 per cent increase for "overtime," for those hours in the day and the night during which he works far past the period of the customary eight hour day.

While the average doctor works hard for what he gets, probably harder than any other one group of individuals, his reward is that he makes enough to live in reasonable comfort in a social environment comparable to that of his clientele. This generalization holds all the way along from the country doctor who is a respected member of his community, whose income is small and expenses light, up to the highly paid specialist. Few physicians succeed in doing more than this. Most die with lamentably small estates.

But it is the exceptional man with an unusually large income such as the $160,000 a year man mentioned above who catches popular fancy and who becomes the subject of a controversy in which unfortunately all the members of his profession are called to account.

It would appear that the occasional very high fees obtained today are not at all unusual in the history of medicine and are quite comparable to ancient experience. The average fees of today are not at all out of proportion to those of

the past. Indeed, this is what one would have anticipated, inasmuch as "physicians' services" is a commodity which has been used or purchased for so many centuries that its average value has been thoroughly standardized. Disproportionate prices may often be obtained for novelties, innovations and luxuries but not for staples. A fictitious value could never have stood so long. Indeed, when one receives his plumber's bill or the electrician's statement one is inclined to marvel at the reasonableness of the physician's charge. Unfortunately the physician is dealing with an intangible service and all that the patient has to show for it afterwards, is more or less return toward health, a condition which he naturally feels should be his anyhow. As with foodstuffs, the patient has used up his commodity as rapidly as purchased. Unlike the case with food, he has been unable to enjoy it for the short time he has had it.

The Committee has been able to supply some very interesting basic observations.

In rural Saskatchewan the financial prospect for physicians is so precarious that several of the rural municipalities or counties have found it necessary to subsidize physicians. The annual salaries of these full time physicians ranged from $3,000 to $5,000. The median salary was $4,000. Many of the physicians earned other incomes from special services. For example one municipal physician employed at an annual salary of $4,000 earned a net income of $4,858.

Here is a section of North America in which a physician is a necessity and in which we may assume that the community is paying as little as it can to hold the doctor. It has found from experience that it must pay from $4,000 to $5,000 or have no doctor. We may therefore take a net income of somewhat over $4,000 as being the actual basic worth of a physician in a civilized frontier community. The better the economic status of the patient, whether he be rural or urban, the more he will insist upon better medical ability and additional services, all of which mean more expense to the doctor. The basic value of the physician to his clientele will therefore increase proportionately with the improved economic status of the clientele.

Or, assuming that the physician's basic value remains the same ($4,000 to $5,000 net) his potentialities for increased income will depend upon his opportunities for increased work. Assuming 300 working days to the year, the municipal physician of Saskatchewan made his basic income from an average of three to five office calls and one to two house visits daily. A physician doing more than this basic amount of work should be able to add the value of the additional work to his basic income.

The Endicott Johnson Corporation, one of the world's largest manufacturers of shoes and tanners of leather, provides a complete medical service for its workers and their families. This service, including hospital facilities, is excellently organized, and in 1927 employed 26 physicians. There were 51 physicians outside this service in the town in which the plant is located.

The net income of physicians employed by the factory range from $3,000 to $12,000. The net income of those in private practice in the same community ranged from under $3,000 to more than $35,000. The analysis of the two sets of incomes is most interesting, showing as it does a distinct parallelism between the two groups, with an important exception: in the group not connected with the

factory there are a few physicians who make very small incomes and a few with relatively large incomes. Neither of these extreme groups appear among the medical employes of the corporation. One might interpret this as indicating that among the outside doctors, those with greatest ability may work harder because of the incentive of larger financial reward, and that those without ability are not being paid more than they are worth. Also, that in the factory group the establishment of a top salary of $12,000 may tend to kill initiative and enthusiasm and a lowest salary of $3,000 may tend to support the laggard.

In the factory group 46.2 per cent of the physicians received net salaries under $5,000. In the outside group 35.3 per cent had net incomes under $5,000. In the factory group 38.4 per cent had incomes between $5,000 and $11,000; in the outside group 41.2 per cent. For incomes between $5,000 and $13,000 the factory group holds 53.8 per cent; the outside group 54.9 per cent. This was a remarkable parallelism. In other words the difference is seen chiefly at the extremes. Nine and eight-tenths per cent of the outside physicians had net incomes under $3,000 while 6 per cent had net incomes over $15,000.

Two apparent facts emerge from these figures, first that independent practice offers the possibility of higher reward for one of unusual ability and second, that the doctors of average ability received comparable incomes in the two groups. There is little likelihood that a great corporation is going to pay a large number of physicians more than it feels they are worth. So, we have another basic value for physicians, this time in industrial communities. The value here ranges from $3,000 to $12,000 net, with 54 per cent of them receiving over $5,000. Most of these are in the $5,000 to $7,000 class. If this latter class is combined with the $3,000 to $5,000 class, we find that 80.8 per cent receive a net income of between $3,000 and $7,000, let us say, average $5,000. This net income of physicians employed by the Corporation is enlarged by certain perquisites such as eligibility to sick benefits, assistance in the purchase of a home, participation in an investment savings organization and old age pensions.

While the figures quoted are those of the Committee on the Costs of Medical Care the interpretations are our own and we realize that others might derive entirely different conclusions from the same figures, but the parallelism between three sets of figures is both striking and instructive. What is the basic value of the ordinary, so-called garden variety of practicing physician, measured in terms of net annual income? Farmers and others living in rural Saskatchewan value his services at between $4,000 and $5,000. A large industrial corporation in New York State places his value around $5,000, actually slightly under this figure. The general practitioners' net income, computed by The American Medical Association is $5,250.

This being the reward for average ability among physicians, a man with exceptional ability has the right to claim a proportionately larger reward if he so desires. The increase is usually obtained from those well able to afford it. Undoubtedly there are individual instances in which the charge is unjustly high but the cause lies as often at the patient's door as at that of the doctor. Who has not heard the patient's anxious relatives urge upon the physician that "he wants the best of everything regardless of the cost" only to be bitterly criticized later by the same individual because of the expense. When an unjust charge has been

made, nine times out of ten it will be rectified by the doctor if his attention is called to it.

From the above review of facts, it would appear that by and large the doctor is not overpaid. In some of the specialties, those branches of medicine in which the method of therapy is impressive or spectacular, the reward is disproportionate to that in other specialties when weighed in terms of ability or services rendered. But even here the figures accumulated by Leland reveal that the average incomes are not exorbitantly high, are in fact pitifully low when compared with those of business executives and others with comparable responsibilities.

We have made out a case for the doctor because he is our present interest. Undoubtedly others could do equally well for the nurse, the hospital and the druggist. If this be true, it would appear that the solution to the problem of the high cost of sickness lies elsewhere. Possibly the most important factor is that in this easy-going country of optimists each person believes that he and his family, contrary to the general experience, will manage to escape the scourge of illness. As in La Fontaine's fable of the grasshopper and the ant, we hop around all summer making no provision for the hardships of the winter.

The problem of the costs of medical care will remain with us until Americans provide for the future instead of living upon the future, or until some form of sickness insurance receives general adoption. By sickness insurance we do not intend to imply the type provided by fraternal organizations, or governmental insurance. The experience of the last few years has amply demonstrated the drawbacks of methods of insurance in which salaried physicians render professional services. Without the personal stimulus of competition, the exercise of ability tends to become sluggish, among doctors equally as much as among all other types of individuals. —*W. T. V.*